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PERSPECTIVES IN RENAL MEDICINE

The concept of glomerular self-defense

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The concept of glomerular self-defense. The balance between local offense factors and defense machinery determines the fate of tissue injury: progression or resolution. In glomerular research, the most interest has been on the offensive side, for example, the roles of leukocytes, platelets, complement, cytokines, eicosanoids, and oxygen radical intermediates. There has been little focus on the defensive side, which is responsible for the attenuation and resolution of disease. The aim of this review is to address possible mechanisms of local defense that may be exerted during glomerular injury. Cytokine inhibitors, proteinase inhibitors, complement regulatory proteins, anti-inflammatory cytokines, anti-inflammatory eicosanoids, antithrombotic molecules, and extracellular matrix proteins can participate in the extracellular and/or cell surface defense. Heat shock proteins, antioxidants, protein phosphatases, and cyclin kinase inhibitors may contribute to the intracellular defense. This article outlines how the glomerulus, when faced with injurious cells or exposed to pathogenic mediators, defends itself via the intrinsic machinery that is brought into play in resident glomerular cells.

In science, a new paradigm may arise from a tiny, unexpected finding that a researcher may confront in a routine experiment. The concept of "glomerular self-defense" occurred to us when we happened to meet an unpredicted experimental outcome.

In the initiation and progression of glomerulonephritis, it has been postulated that various cytokines play crucial roles. Once inflammation is initiated, glomeruli primed by infiltrating leukocytes may become more susceptible to injury by inflammatory cytokines. To examine this possibility, we tested the cytokine responses of normal and nephritic glomeruli using isolated glomeruli. As expected, when normal glomeruli were stimulated with a proinflammatory cytokine interleukin (IL)-1β, the expression of the cytokine-responsive metalloproteinase stromelysin was induced. Unexpectedly, however, the IL-1–triggered gene expression was blunted in glomeruli in the regeneration phase of acute glomerulonephritis [1]. The blunted response was observed not only for stromelysin but also for other cytokine-inducible molecules. Furthermore, when activated macrophages were adoptively transferred into the nephritic glomeruli, the induction of inflammatory mediators in resident cells was suppressed, compared with the induction that occurred in normal glomeruli (abstracts; Ogura et al, *J Am Soc Nephrol* 8:487A, 1997; Sütö and Kitamura, *J Am Soc Nephrol* 8:481A, 1997) [1, 2]. We hypothesized that after an inflammatory insult, the glomerulus may acquire the potential for protecting itself from further activation and injury.

The idea of glomerular self-defense has not been addressed before, but the enhancement of local tissue defense in response to environmental stresses is a well-known phenomenon in certain pathophysiological situations. The best-known example is "ischemic preconditioning," in which a brief period of ischemia results in the resistance of tissues to subsequent, severe ischemia. In the kidney, ischemic preconditioning prevents the reduction in glomerular filtration rate caused by ischemia/reperfusion or intra-arterial infusion of hydrogen peroxide. Another example of tissue defense is "thermotolerance." In various tissues and cultured cells, the exposure to thermal stress induces a set of stress proteins, so-called heat shock proteins (HSPs), thereby affording tolerance against subsequent insults. It is therefore not surprising that the glomerulus, within inflammatory milieus, acquires an insensitivity to stimuli, although the acquisition of the "anti-inflammatory status" by inflamed organs or tissues is not well addressed to date. The aim of this review is to address possible mechanisms of local defense that may be exerted during glomerular injury. This article outlines how the glomerulus, when faced with injurious cells or exposed to pathogenic substances, defends itself via the intrinsic machinery that is brought into play in resident cells, especially in mesangial cells.

Key words: glomerulonephritis, cytokine inhibitor, ani-inflammatory cytokine, antioxidant, heat shock protein, cyclin kinase inhibitor.

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GENERAL PRINCIPLES AND CLASSIFICATION OF DEFENSE SYSTEMS

Many acute inflammations resolve without specific therapy. Like the initiation of inflammation, recovery from

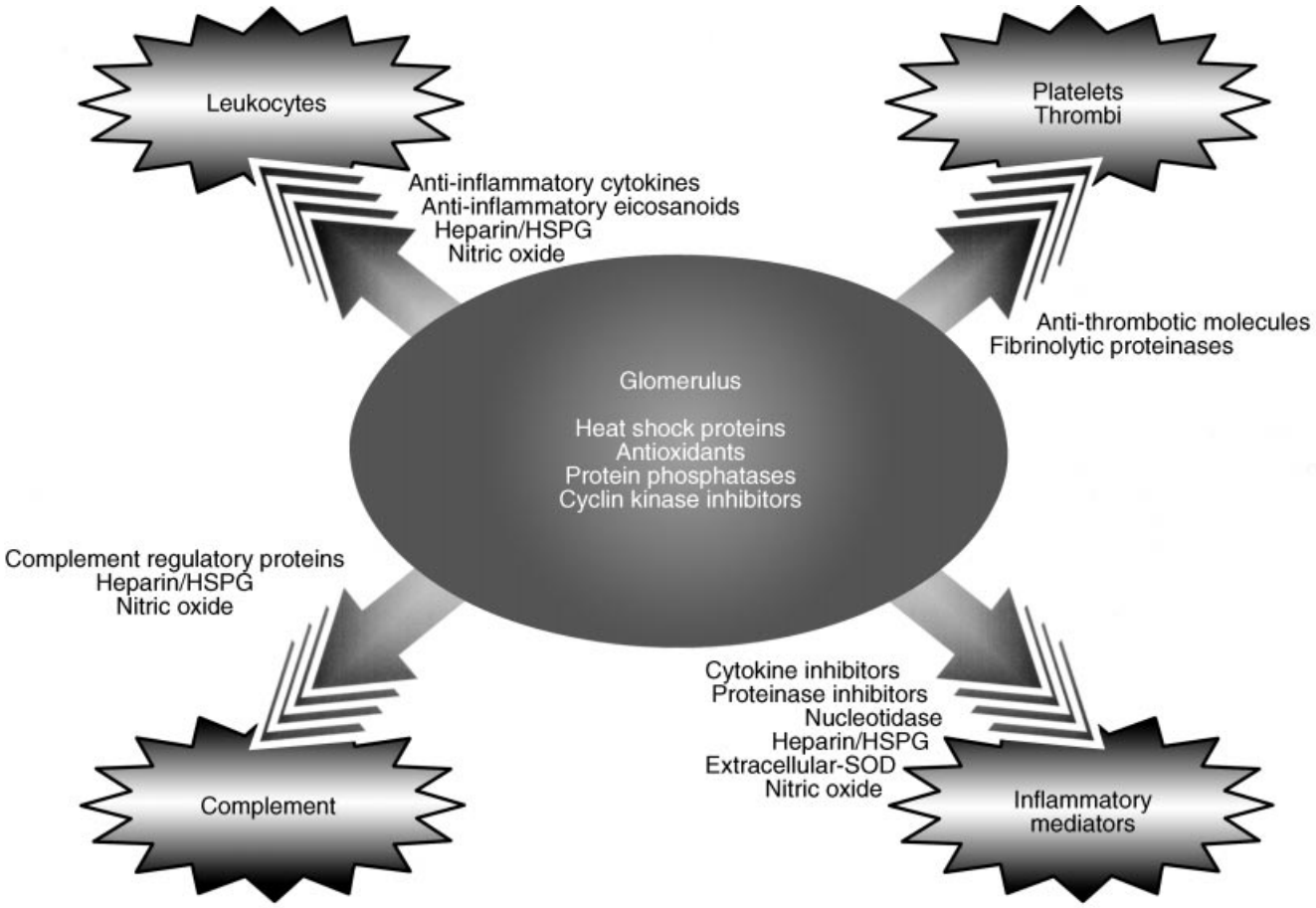

Fig. 1. Glomerular defense mechanisms: Defenders and their targets. Abbreviations are: HSPG, heparan sulfate proteoglycan; SOD, superoxide dismutase.

inflammatory disease is a dynamic, multifaceted process in which a number of anti-inflammatory molecules are elaborated under well-ordered, switch-off programs. Figure 1 outlines glomerular defense against leukocytes, platelets/thrombi, and complement and inflammatory mediators. As illustrated, the vast array of factors can be mobilized in defense of the glomerulus, and its distribution and regulation is listed in Tables 1 and 2. A brief overview of the defense strategies is pertinent.

Toward resolution of inflammation, the defending machinery counteracts the attack by offenders at multiple levels, that is, extracellular, cell surface, and intracellular levels. For example, activated resident cells produce inactivators for leukocytes or platelets and inhibitors for specific mediators, including cytokines/growth factors, oxidants, proteinases, coagulants, and eicosanoids. A number of defending molecules are involved in this "extracellular defense." Complement-mediated injury is another important mechanism involved in the generation of glomerular disease. Glomerular cells have cell membrane-bound complement inhibitors that participate in the "cell-surface defense." In response to environmental perturbation, resident cells produce intracellular stress proteins that reinforce cellular resistance to subsequent stresses. HSPs and antioxidant enzymes are involved in this "intracellular defense." In addition, several "self-cooling" devices are present in resident cells. These include the production of autocrine inactivators, the creation of new extracellular matrix (ECM), and the induction of protein phosphatases and cyclin kinase inhibitors (CKIs).

Endogenous defending molecules can be classified in different ways, based on the range of their targets and kinetics under pathophysiological situations. Some defending molecules have very specific targets. Thus, cytokine inhibitors, proteinase inhibitors, complement regulatory proteins, antithrombotic molecules, antioxidants, and CKIs are the typical examples of "man-to-man combat." On the other hand, other defending molecules have a wide range of targets. This may be viewed as "shotgun defense," which involves anti-inflammatory cytokines, anti-inflammatory eicosanoids, heparin/heparan sulfate proteoglycan (HSPG), and nitric oxide (NO). In pathological situations, the defense system is more complicated. Various defending molecules may form cascades

Table 1. Distribution of glomerular defenders

Defending molecule	Producer/Distribution					
	Mesangial cells	Epithelial cells	Endothelial cells	Leukocytes	GBM	Undetermined
Extracellular/cell-surface defense						
Cytokine inhibitors						
IL-1ra				○		▲
sTNFR				○		▲
SPARC	○▲	●				
decorin	○●					
Protein inhibitors						
TIMP	○					●
crystatin	○					
Complement inhibitors						
DAF	○▲					●
CR1		○●				
MCP	○●	○●	●			
CD59	○●	○●	○●			
Crry	○●	○	○●			
C1 inhibitor		○				
factor H	○●					
vitronectin	●		▲		●	
clusterin	○●	○●				
Nucleotidase						
ATP/ADPase					●	
Anti-inflammatory cytokines						
TGF-β1	○▲	○		○		●
IL-4	▲	▲		○▲		
IL-6	○●	○		○		
IL-10	○			○		▲
IL-13				○		▲
LIF	○			○		●
Anti-inflammatory eicosanoids						
postaglandin E_2	○●	○	○			
prostaglandin I_2	○	○	○			
lipoxin A_4	○					▲
lipoxin B_4	○					
Anti-thrombotic molecules						
thrombomodulin	○●		●			
tissue factor pathway inhibitor	○					●
tissue-type PA	○	○	●			
urokinase-type PA		○●				
Extracellular matrix						
mesangial matrix	○●					
heparin/heparin sulfate proteoglycan	○●	○	○●		●	
Antioxidant						
extracellular-superoxide dismutase			●			
Other						
nitric oxide	○		●	○		
Intracellular defense						
Heat shock proteins						
HSP90		●				
HSP70	○	●				
HSP60						
HSP27	●					
heme oxygenase-1	○					
Antioxidants						
superoxide dismutases	○	○	○			●
catalase			○			●
glutathione peroxidase						●
Protein phosphatases						
MKP-1	○▲					
PAC1	○					
B23	○					
PTPγ		●				
RPTP-BK		●				
GLEPP1		●				
Cyclin kinase inhibitors						
p21		▲				
p27	○●	▲				

Symbols are: ○, expression/production in culture; ●, distribution in normal glomeruli; ▲, induction in diseased glomeruli.

Abbreviations are: GBM, glomerular basement membrane; IL-1ra, IL-1 receptor antagonist; sTNFR, soluble tumor necrosis factor receptor; SPARC, secreted protein acidic and rich in cysteine; TIMP, tissue inhibitor of metalloproteinases; DAF, decay accelerating factor; CR1, complement receptor 1; MCP, membrane cofactor protein; Crry, complement receptor related gene y; ATP/ADPase, adenosine triphosphatase and adenosine diphosphatase; TGF-β1, transforming growth factor-β1; LIF, leukemia inhibitory factor; PA, plasminogen activator; HSP, heat shock protein; MKP-1, MAP kinase phosphatase 1; PTPγ, protein-tyrosine phosphatase-γ; RPTP-BK, receptor-type protein-tyrosine phosphatase in the brain and kidney; GLEPP1, glomerular epithelial protein 1; p21, $p21^{Cip1/Waf1/Sdi1/Cap20}$; p27, $p27^{Kip1}$.

Table 2. Regulation of glomerular defenders

Defending molecule	In vitro stimuli: Up-regulation	In vitro stimuli: *Down-regulation*	In vivo situations: Up-regulation	In vivo situations: *Down-regulation*
Extracellular/cell-surface defense				
Cytokine inhibitors				
IL-1ra	LPS, IL-4, TGF-β (Mo/MΦ)		α-GBM	
sTNFR	LPS (Mo)		human GN	
SPARC	PDGF, EGF (Mes)		Heymann (Epi), α-Thy 1 (Mes)	
decorin	TGF-β (Mes)		α-Thy 1 (Mes)	
Proteinase inhibitors				
TIMP	IL-1β, TNF-α, HG, TGF-β (Mes)		human GN [IgA, DN], PAN	
Complement inhibitors				
DAF	complement (Mes)		human GN [IgA, MN, FGS, MPGN, HUS, SLE, crescentic] (Mes)	
CRI			*human GN [MN, DN, IgA, SLE] (Epi)*	
MCP	complement (Mes)		human GN [MPGN, SLE, IgA] (End, Mes)	
CD59	complement (Mes) IL-1β, TGF-β (Epi)		human GN [SLE] (End), *human GN [MN]*	
C1 inhibitor	IFNγ (Epi)			
factor H	IFNγ (Mes)			
vitronectin			human GN [IgA, HUS] (Mes), [MN] (End)	
clusterin	thrombin (Mes, Epi)		human GN [MN, etc.], Heymann	
Nucleotidase				
ATP/ADPase			*endotoxemia, α-Thy 1, α-GBM*	
Anti-inflammatory cytokines				
TGF-β1	Ang II, ANP, thrombin, TX HG, IC, stretch (Mes)		α-Thy 1, α-GBM, Habu, PAN, DN human GN [IgA, FGS, SLE, DN, crescentic]	
IL-4	HG, oxiLDL (Epi)		α-GBM, human GN [IgA, SLE, MN, MCD]	
IL-6	LPS, IL-1β, TNF-α, GIP, Ang II, IC (Mes) LPS, IL-1β, TNF-α (Epi)		human GN [IgA, IgM, DN]	
IL-10			α-GBM	
IL-13			α-GBM	
LIF	LPS, IL-1β, TNF-α, PDGF (Mes)		α-GBM	
Anti-inflammatory eicosanoids				
prostaglandin E_2	IL-1β, TNF-α, IL-6, PDGF, Ang II, AVP ET-1, thrombin, ROI, NO, IC, HG (Mes) AVP (Epi)		α-GBM, Heymann	
prostaglandin I_2	IL-1β, TNF-α (Mes) AVP (Epi)			
lipoxin A_4			α-GBM, ConA/α-ConA	

or networks. That is, one defending molecule triggers the expression of another or synergistically cooperates with others against offenders. The "defense cascade" or "defense network" may be important for the defense programs of the glomerulus.

Many defending molecules are inducible in response to a local inflammatory milieu. This "inducible defense" contributes to the resolution of established inflammation. In contrast, some defending molecules are constitutively expressed and/or are present in the glomerulus. This "constitutive defense" is conceivably involved in the prevention of injury and the maintenance of integrity of the glomerulus. For example, complement regulatory proteins and heparin/HSPG may function as the constitutive defenders. In both inducible and constitutive examples, defending molecules are produced by resident glomerular cells. Alternatively, these molecules may be adoptively transferred from other sites via the circulation and may be stored within the glomerulus. This "adoptive defense" should also be considered in the pathophysiological contexts.

SELF-DEFENSE MACHINERY IN THE GLOMERULUS

Extracellular defense

Inhibitors of inflammatory mediators

During inflammation, a number of extracellular mediators are released from infiltrating cells and resident cells, leading to the magnification of inflammatory processes. However, in many cases, inflammation is self-limiting. This is due to intrinsic mechanisms designed to control inflammatory processes. Both resident and infiltrating cells have the ability to elaborate a set of natural inhibitors against specific, pathogenic mediators. This "man-to-man combat" system is widely observed in mammals and plays an important role in the prevention and resolution of inflammation. This section summarizes the potential roles of natural inhibitors that regulate the activity of pathogenic substances in the glomerulus.

(1) Inhibitors of cytokines and growth factors

(a) Interleukin-1 receptor antagonist (IL-1ra). Interleukin-1 is a proinflammatory cytokine that plays a crucial

Table 2. *Continued*

Defending molecule	In vitro stimuli: Up-regulation *Down-regulation*	In vivo situations: Up-regulation *Down-regulation*
Anti-thrombotic molecules		
thrombomodulin	*LPS, TNF-α (isol.G)*	human GN [MPGN, SLE]
tissue factor pathway inhibitor	heparin (Mes)	α-*GBM*
tissue-type PA	IL-1β (Mes) IL-1β, TNF-α, thrombin (Epi)	α-*GBM,* human GN [IgA, SLE]
urokinase-type PA	TNF-α, thrombin (Epi)	*human GN [SLE]*
Extracellular matrix		
mesangial matrix	IL-1β, TNF-α, TGF-β, HG, TX Ang II, AVP, oxiLDL, stretch (Mes)	various human and experimental GN
heparin/heparan sulfate proteoglycan	TGF-β, *Ang II, HG* (Mes)	α-Thy 1, PAN, renal ablation
Other		
nitric oxide	LPS, IL-1β, TNF-α, IFNγ (Mes) *TGF-β, PDGF, MP, PGE_2 (Mes)* bradykinin, ATP, thrombin, PAF (End)	α-Thy 1, α-GBM, Heymann, *in situ* GN
Intracellular defense		
Heat shock proteins		
HSP70	*IL-1β (Mes)*	PAN, α-*GBM*
HSP27		PAN (Epi)
heme oxygenase-1	IL-1β (Mes)	
Antioxidants		
superoxide dismutase	IL-1β, ROI, heat-aggregated IgG, MP (Mes) IL-1α, LPS, ROI (Epi) ROI, MP (End)	injection of ROI, MP
catalase	MP (End)	injection of ROI, MP
glutathione peroxidase		injection of ROI, MP
Protein phosphatases		
MKP-1	ROI (Mes)	α-Thy 1, α-GBM (Mes)
GLEPP1		α-*GBM (Epi)*
Cyclin kinase inhibitors		
p21		α-Thy 1 (Mes), Heymann (Epi)
p27	TGF-β, *PDGF, bFGF* (Mes)	α-*Thy 1 (Mes)*, Heymann (Epi)

Abbreviations are: LPS, lipopolysaccharide; Mo/MΦ, monocytes/macrophages; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; Mes, mesangial cells; HG, high glucose concentration; Epi, glomerular epithelial cells; IFNγ, interferon-γ; Ang II, angiotensin II; ANP, atrial natriuretic peptide; TX, thromboxane; oxiLDL, oxidized low density lipoprotein; GIP, granulocyte inhibitory protein; IC, immune complex; AVP, arginine vasopressin; ET-1, endothelin-1; ROI, reactive oxygen intermediates; NO, nitric oxide; isol.G, isolated glomeruli; MP, methylpredonisolone; PAF, platelet activating factor; End, glomerular endothelial cells; bFGF, basic fibroblast growth factor; α-GBM, anti-glomerular basement membrane nephritis; GN, glomerulonephritis; Heymann, Heymann nephritis; α-Thy 1, anti-Thy 1 glomerulonephritis; IgA, IgA nephropathy; DN, diabetic nephropathy; PAN, puromycin aminonucleoside nephrosis; MN, membranous nephropathy; FGS, focal/segmental glomerulosclerosis; SLE, lupus nephritis; MPGN, membranoproliferative glomerulonephritis; HUS, hemolytic uremic syndrome; crescentic, crescentic glomerulonephritis; Habu, Habu-venom-induced glomerulonephritis; MCD, minimal change disease; IgM, IgM nephropathy; ConA/α-ConA, concanavalin A/anti-concanavalin A immune complex glomerulonephritis; *in situ* GN, *in situ* immune complex glomerulonephritis. Producers are in parentheses.

role in a wide range of inflammatory disorders. In glomerulonephritis, IL-1 has been regarded as one of pivotal pathogenic mediators. IL-1, released by inflammatory cells, causes resident cells to proliferate and stimulate aberrant matrix metabolism, expression of adhesion receptors, and the release of inflammatory mediators, including cytokines/growth factors, chemokines, bioactive lipids, metalloproteinases, and reactive oxygen/nitrogen species [3, 4]. *In vivo*, a number of studies have suggested a role for local IL-1 in the pathogenesis of experimental and human glomerulonephritis [4].

Interleukin-1 receptor antagonist is a naturally occurring IL-1 inhibitor originally identified as a protein secreted from myelomonocytic cells. This molecule is structurally homologous to IL-1α and IL-1β and competes with IL-1 for binding to IL-1 receptors type I and II [5]. The therapeutic utility of IL-1ra has been reported in various experimental diseases [6]. In the kidney, systemic administration of IL-1ra suppresses: glomerular cell proliferation, the expression of macrophage inflammatory protein-2 (MIP-2) and intercellular adhesion molecule-1 (ICAM-1), the formation of intracapillary thrombi, leukocyte infiltration (neutrophils, monocyte/macrophages, and/or lymphocytes), and proteinuria in anti-glomerular basement membrane (GBM) nephritis and anti-Thy 1 glomerulonephritis in rats [7–12]. A similar therapeutic effect was reported by the administration of soluble IL-1 receptor [10, 11]. Treatment with IL-1ra may be effective even after the establishment of chronic glomerulonephritis. Lan et al reported that the administration of IL-1ra halted the progression of established anti-GBM glomerulonephritis [13].

The expression of IL-1ra is induced in various inflammatory diseases. In normal rat glomeruli, the expression of IL-1ra is not detectable [14]; however, obvious up-regulation of IL-1ra is observed in glomeruli subjected to anti-GBM nephritis [14]. Currently, the cell type responsible for the expression is not determined. One pos-

sible candidate is inflammatory leukocytes [14] because neutrophils and monocyte/macrophages produce IL-1ra *in vitro*. The production of IL-1ra by resident glomerular cells should not be excluded because this molecule is also produced by nonleukocytic cells. Of note, the promoter region of the IL-1ra gene contains the nuclear factor-κB (NF-κB) binding site that may be activated in response to inflammatory stimuli [15]. Indeed, the expression of IL-1ra is up-regulated by IL-1, IL-4, IL-6, transforming growth factor-β (TGF-β), and lipopolysaccharide (LPS) in monocyte/macrophages and hepatocytes [15–18]. Possibly, both resident and infiltrating cells produce IL-1ra, and locally produced IL-1ra may contribute to limiting the severity of glomerulonephritis.

(b) Soluble tumor necrosis factor (TNF) receptor (sTNFR). Similar to IL-1, TNF-α is a proinflammatory cytokine involved in the pathogenesis of glomerular diseases, including puromycin aminonucleoside (PAN) nephrosis, murine models of lupus nephritis, serum sickness nephritis, anti-GBM nephritis, and antineutrophil cytoplasmic autoantibodies-positive human glomerulonephritis [19]. TNF-α, which is mainly produced by infiltrating leukocytes, causes resident cells to express adhesion receptors for leukocytes and release of inflammatory mediators [19]. The blockade of the TNF action via neutralizing antibodies attenuates experimental glomerulonephritis [20, 21], suggesting its pathological contribution.

Soluble tumor necrosis factor receptors (sTNFRp55 and sTNFRp75) are naturally occurring TNF inhibitors, which are truncated forms of cell surface TNF-α receptors. These molecules, originally found in the plasma of clinical and experimental endotoxemia, neutralize TNF-α–induced cytotoxicity and immunoreactivity *in vitro*. The therapeutic utility of sTNFRs for glomerulonephritis has been reported by several investigators. For example, the systemic administration of sTNFR preserved renal function and suppressed histopathologic changes (intracapillary thrombi and crescent formation), expression of MIP-2 and macrophage migration inhibitory factor (MIF), neutrophil infiltration, and proteinuria in anti-GBM nephritis in rats [10, 11, 20, 22].

Urine of patients with chronic glomerulonephritis exhibits the TNF inhibitory activity. Suzuki et al identified that these inhibitors are sTNFRp55 and a homologue of sTNFRp75 [23]. This evidence raises possibilities that sTNFRs may be produced in nephritic glomeruli and that locally produced sTNFRs might contribute to limiting the action of TNF-α in the glomerulus. It has been shown that peripheral blood monocytes release sTNFR in response to LPS [24]. It has not been determined whether resident glomerular cells contribute to the production of sTNFR in nephritic glomeruli.

(c) Secreted protein acidic and rich in cysteine (SPARC). Platelet-derived growth factor (PDGF) is a prototypical growth factor that plays an important role in various pathological situations. PDGF is expressed in a wide range of experimental and human glomerular diseases [25]. PDGF induces proliferation of mesangial cells *in vitro* and *in vivo* [25]. In anti-Thy 1 glomerulonephritis in rats, the inhibition of PDGF by the administration of an anti-PDGF neutralizing antibody resulted in the attenuation of cell proliferation and matrix expansion, suggesting the crucial role of PDGF in mesangial proliferative glomerulonephritis [26].

Secreted protein acidic and rich in cysteine, also known as osteonectin, is a glycoprotein produced by various cells. SPARC interacts with PDGF-BB and PDGF-AB and thereby inhibits their binding to PDGF receptors [27]. *In vitro*, the expression of SPARC is induced by growth factors including PDGF, TGF-β, and insulin-like growth factor-I [28], suggesting possible up-regulation of this molecule under pathologic circumstances.

In the normal rat glomerulus, SPARC is constitutively expressed in glomerular epithelial cells [29]. This expression is increased in passive Heymann nephritis [29]. In contrast, after the induction of anti-Thy 1 glomerulonephritis, the expression of SPARC is induced in mesangial cells [30]. Cultured mesangial cells express and secrete SPARC, and the production is enhanced by PDGF and epidermal growth factor (EGF), the putative pathogenic mediators for anti-Thy 1 glomerulonephritis [30]. When SPARC is added externally, the DNA synthesis of mesangial cells is inhibited. Based on the role of PDGF in mesangial cell proliferation and matrix expansion *in vivo* [26], endogenous SPARC may be involved in self-defense of the glomerulus against inflammation.

(d) Decorin. Excessive deposition of ECM is the characteristic feature of tissue fibrosis. Numerous reports have suggested a role for TGF-β in the fibrogenesis of organs. In glomerular disease, TGF-β is up-regulated under various pathologic situations and has been regarded as a prosclerotic mediator [31]. TGF-β induces the deposition of ECM by stimulating matrix production, decreasing ECM-degrading proteinases, and up-regulating proteinase inhibitors [32]. Although the role of TGF-β in glomerular disease is not fully elucidated, these data suggest a possible contribution of TGF-β to the expansion of ECM in nephritic glomeruli. Indeed, the administration of an anti–TGF-β neutralizing antibody or an antisense TGF-β oligodeoxynucleotide attenuated accumulation of ECM in acute anti-Thy 1 glomerulonephritis [33, 34].

Decorin is a proteoglycan that binds TGF-β1, TGF-β2, and TGF-β3 and, thereby, neutralizes their biological activities [35]. Border et al reported that systemic injection of recombinant decorin inhibited matrix expansion and proteinuria in acute anti-Thy 1 glomerulonephritis in which TGF-β plays an important role [35]. Similarly, intramuscular transfer of a decorin gene effectively sup-

pressed the accumulation of glomerular ECM in the same experimental model [36].

Cultured mesangial cells constitutively produce decorin, and its production is up-regulated after exposure to TGF-β [37]. Expression of decorin is induced in glomeruli in parallel with TGF-β expression in anti-Thy 1 glomerulonephritis [38]. These data suggest a possibility that decorin is involved in an autoregulatory mechanism that limits excessive action of TGF-β during acute glomerulonephritis.

(2) Proteinase inhibitors

(a) Tissue inhibitor of metalloproteinases (TIMP). Matrix metalloproteinase (MMP) is a family of enzymes that degrades ECM components. The MMP family includes interstitial collagenases, gelatinases, stromelysins, and punctuated metalloproteinases (PUMPs) and plays an important role in the normal turnover of ECM. Aberrant activity of MMP is associated with various pathologies, including tissue fibrosis, inflammation, tumor invasion/metastasis, and atherosclerosis [39].

In the normal glomerulus, certain MMPs are constitutively expressed [40]. The glomerular expression of MMPs, including gelatinase A, gelatinase B, stromelysin-1, and PUMP-1, is increased under pathologic situations such as anti-Thy 1 glomerulonephritis, PAN nephrosis, passive Heymann nephritis, diabetic nephropathy, and several human glomerulonephritis (abstract; Miyazaki et al, *J Am Soc Nephrol* 6:902, 1995) [41–45]. The cells responsible for the production of MMPs are resident cells and inflammatory leukocytes [46]. Elaborated MMPs contribute to structural alteration of the mesangial matrix and GBM [40, 42, 47], leading to proteinuria [48].

Recent investigations disclosed a role of MMPs in the regulation of glomerular cell function, especially mesangial cell proliferation. The inhibition of constitutive expression of gelatinase A in mesangial cells led to depressed mitogenesis [49, 50]. Overexpression of stromelysin-1 in mesangial cells caused enhanced mitogenesis and migration within ECM [51]. These results suggest that certain MMPs function as autocrine triggers for mesangial cell activation. Potential mechanisms involved in the cellular activation by MMPs were reviewed previously [52].

From the viewpoint described above, the inhibition of excessive MMPs is beneficial for the attenuation of glomerular disease. The activity of MMPs is tightly regulated by their endogenous, specific inhibitors, TIMPs [39]. TIMP-1 and TIMP-2 are constitutively expressed and/or are secreted by cultured mesangial cells and isolated normal glomeruli [53, 54]. The expression of TIMP-1 in mesangial cells is up-regulated by inflammatory cytokines, including IL-1β and TNF-α [55], TGF-β [56], or certain metabolic stimuli such as high glucose concentrations [57]. TIMP-1 is up-regulated in glomeruli of diabetic nephropathy and PAN nephrosis [42, 58]. TIMPs expressed in glomeruli may function as endogenous defenders that protect glomeruli from MMP-mediated injury.

(b) Cystatin. Cysteine proteinase is a diverse family of lysosomal enzymes with broad substrate specificity. These enzymes, including cathepsins B, H, and L, are generally located in lysosomes. However, a number of normal and tumor cells secrete these enzymes into the extracellular space. Although cysteine proteinases function optimally at acid pH values, they may have extracellular activity in acidic microenvironment. It has been shown that cathepsins B, H, and L are normally present in rat glomeruli [46]. These enzymes are also secreted by activated macrophages and neutrophils [59, 60], the major effector cells in glomerular disease. Endogenous cysteine proteinases present in glomeruli degrade intact GBM [61]. A role of cysteine proteinases in the pathogenesis of glomerular inflammation has been indicated by some investigators. The administration of specific inhibitors of cysteine proteinases (E-64, Ep475) reduced proteinuria in anti-GBM glomerulonephritis in rats. This therapeutic effect was correlated with a marked decrease in the local activity of cathepsins B and L [62, 63].

The activity of cysteine proteinases is inhibited by naturally occurring inhibitors, including cystatins [64]. The expression of cystatin C, the major member of the cystatin superfamily found in body fluid, is observed in various human tissues, including the kidney [65]. It has been reported that cystatin C is secreted by cultured mesangial cells [66]. Locally produced cystatin C could be involved in the protection of glomeruli from cysteine proteinase-mediated injury.

(3) Complement regulatory proteins

Complement is a major mediator of tissue injury in various glomerulonephritis. Activation of complement occurs through the classical pathway and the alternative pathway; the former is triggered by antibody binding to antigens, and the latter is directly activated by microorganisms and damaged cells. The activation of complement attracts inflammatory cells and triggers resident cells to express adhesion receptors and to produce inflammatory mediators, including reactive oxygen intermediates (ROI), proteinases, and cytokines/chemokines, leading to glomerular injury [67]. The activation of complement is controlled by endogenous regulatory proteins present in plasma (C1 inhibitor, C4-binding protein, factor H, factor I, vitronectin, clusterin) and on the cell surface [decay accelerating factor (DAF), membrane cofactor protein (MCP), complement receptor 1 (CR1), membrane attack complex (MAC) inhibitory factor, complement receptor-related gene y (Crry)]. Complement regulatory proteins are normally present in the glomerulus, and under pathological circumstances, local levels of these proteins alter [68]. The functional inhibition of these molecules exacerbates pathological processes, indicating crucial roles for complement regulatory pro-

teins in the protection of glomeruli from antibody/complement-mediated injury.

(a) Decay accelerating factor. Decay accelerating factor inhibits complement activation by intervention in C3/C5 convertases via removal of C2b from C4b and Bb from C3b. DAF is a membrane-bound protein and is anchored to the plasma membrane by a carboxy terminal glycosyl-phosphatidylinositol (GPI). DAF is present in normal human glomeruli [69]. Modest expression of DAF mRNA may be detectable in normal glomerular cells [70]. In human glomerular disease, DAF is frequently detected in the mesangium, where C1q and C3 are colocalized [70, 71]. Using *in situ* hybridization, Abe et al reported that DAF mRNA is up-regulated in mesangial cells in IgA nephropathy and membranous nephropathy [70]. *In vitro*, mesangial cells have the ability to produce functional DAF, and the production and membrane expression are up-regulated by terminal complement components [72]. Antibody-mediated inhibition of DAF in glomerular cells enhances susceptibility to complement-induced cytotoxicity [69]. A recent study using *ex vivo* perfusion of human DAF-transgenic pig kidneys with human blood revealed that overexpression of DAF is sufficient to inhibit complement activation on the glomerular endothelium [73].

(b) Complement receptor 1. Similar to DAF, CR1 inactivates C3b and C4b. CR1 also functions as a cofactor for factor I, a plasma serine proteinase that cleaves C3b and C4b. In the glomerulus, CR1 is present exclusively on the surface of podocytes [68]. Consistent with this, glomerular epithelial cells in culture produce CR1 [74]. The expression of CR1 in podocytes is decreased in certain glomerular diseases, including membranous nephropathy, diabetic nephropathy, IgA nephropathy, and diffuse proliferative lupus nephritis [75–77]. The administration of a soluble form of CR1 lacking transmembrane and cytoplasmic domains significantly reduced both histopathologic and functional consequences of anti-Thy 1 glomerulonephritis, concanavalin A/anticoncanavalin A glomerulonephritis, and passive Heymann nephritis in rats [78].

(c) Membrane cofactor protein. Membrane cofactor protein (MCP) is a type 1 membrane glycoprotein that serves as a cofactor for factor I in inactivating C3b and C4b deposited on the cell membrane. This inactivation of C3b and C4b by MCP is associated with the protection of cells from autologous complement attack. In the normal human glomerulus, MCP is present on the surface of endothelial, mesangial, and epithelial cells [68]. Consistent with this, glomerular epithelial and mesangial cells in culture produce MCP [79]. The expression of MCP is up-regulated by activated complement in cultured mesangial cells [80]. MCP is increased in the glomerular capillary and mesangium of human kidney diseases, including membranoproliferative glomerulonephritis, IgA nephropathy, and lupus nephritis [81]. Currently, the *in vivo* function of MCP is not fully determined.

(d) CD59. Like DAF, CD59 (also named MAC, protectin, or homologous restriction factor-20) is another GPI-anchored protein that inhibits the incorporation of C9 into C5b-9 complexes. In the normal glomerulus, CD59 is present on the surface of endothelial, mesangial, and epithelial cells [68]. Cultured glomerular epithelial, mesangial, and endothelial cells express CD59 [82, 83]. The expression of CD59 is up-regulated in glomerular epithelial cells by IL-1β and TGF-β [84] and in mesangial cells by activated complement [85]. When treated with anti-CD59 antibody, these cells become more susceptible to complement-mediated lysis *in vitro* [82, 83]. In contrast, mesangial cells transfected with a CD59 cDNA show remarkable resistance to both lytic complement attack and sublytic effect of C5b-9 [86]. The expression of CD59 in the glomerular capillaries is increased in diffuse lupus nephritis [87]. In contrast, glomerular CD59 is decreased in membranous nephropathy [88].

In vivo roles of CD59 have been examined by several investigators. Matsuo et al examined the effect of anti-CD59 monoclonal antibody in a rat model of complement-dependent glomerulonephritis [89]. They found that local inhibition of endogenous CD59 enhanced glomerular cell proliferation, ICAM-1 expression, leukocyte influx, and fibrin deposition. Nangaku et al examined the effect of anti-CD59 antibody in the pathogenesis of thrombotic microangiopathy induced by an antibody to endothelial cells [90]. They found that local neutralization of CD59 exacerbated the disease, that is, more glomerular C5b-9 formation, endothelial damage, and platelet/fibrin deposition, resulting in the deterioration of renal function. These *in vivo* data clearly evidenced that CD59 is an endogenous defender against complement-mediated glomerular injury.

(e) Complement receptor-related gene y. In humans, DAF and MCP work at the step of C3/C5 convertases. Crry is the rodent analogue that possesses the function of DAF and MCP. In the normal rat glomerulus, Crry is present on the surface of mesangial and endothelial cells [68]. *In vitro*, all of the glomerular epithelial, mesangial, and endothelial cells produce Crry [82, 83]. When treated with an anti-Crry antibody, these cells become more susceptible to complement-mediated cytotoxicity [82, 83]. Furthermore, mesangial cells overexpressing Crry exhibit resistance to complement-mediated cell lysis and to sublytic stimulatory effects of complement [91].

In vivo roles of Crry have been investigated by a few investigators. Using a monoclonal antibody against Crry, Matsuo et al showed that Crry plays a vital role by preventing complement activation on the vascular endothelium [92]. Using the same strategy, they also showed that local inhibition of Crry resulted in accelerated mesangio-

lysis and leukocyte influx in anti-Thy 1 glomerulonephritis [93].

(f) C1 inhibitor. C1 inhibitor binds to C1 and C1r:C1s and releases them from C1q. It has been shown that glomerular epithelial cells in culture produce C1 inhibitor and that its production is enhanced by interferon-γ [94]. The distribution of this molecule in the normal glomerulus remains to be determined.

(g) Factor H. Factor H binds to C3b and exhibits decay-accelerating activity and cofactor activity. The crucial role of this molecule in the maintenance of glomerular integrity was supported by evidence that inherited deficiency of factor H results in glomerulonephritis in pigs and humans [95, 96]. In normal human kidneys, factor H is localized in the mesangium [97]. Consistent with this, cultured mesangial cells produce factor H, and its production is enhanced by interferon-γ [98].

(h) Vitronectin. Vitronectin, also called S-protein, is a component of ECM. This molecule binds to C5b-7 and inhibits the action of MAC. In the normal human glomerulus, vitronectin is present in the mesangium and GBM [99, 100]. Currently, it is not known whether the normal distribution of vitronectin is due to local production by glomerular cells or by the deposition of plasma protein. In glomerulonephritis, vitronectin is abundantly present in the immune deposit of the glomerular capillaries and mesangium and is colocalized with C3b and C5b-9 [99, 100].

(i) Clusterin. Human clusterin (SP-40,40) was first identified in glomerular immune deposits of a patient with membranous nephropathy [101]. Clusterin is part of the fluid phase MAC and functions as a complement inhibitor [102]. This molecule binds to C3, C8, and C9, inhibits generation of MAC, and thereby attenuates complement-mediated cytolysis. In a number of immune-mediated glomerulonephritis, clusterin is found in the glomerulus in conjunction with components of MAC [103–105].

Laping et al reported that clusterin mRNA and protein are detectable in normal rat glomeruli [106]. Clusterin mRNA is constitutively expressed in cultured podocytes and mesangial cells, and the expression is up-regulated by certain stimuli, including thrombin [106]. Using kidneys subjected to complement-dependent, passive Heymann nephritis, Saunders et al reported that the isolated kidneys perfused with clusterin-depleted plasma developed increased proteinuria, accelerated deposition of terminal complement components, and enhanced podocyte injury in the glomerulus [107]. This result indicates a possible role for local clusterin in the protection of glomeruli from complement-mediated injury.

(4) Oxygen radical scavengers

Extracellular superoxide dismutase (EC-SOD). Superoxide dismutases are mainly localized in the intracellular compartment but are also present in extracellular fluid at low concentrations [108]. EC-SOD is a secretory glycoprotein that is the major SOD isozyme in extracellular fluids. The EC-SOD binds to heparan sulfate proteoglycan (HSPG) on endothelial cells and functions as a "protective coat" that attenuates oxidant-mediated cell injury [109].

(5) Inhibitors of adenine nucleotides

Adenosine triphosphatase and adenosine diphosphatase (ATP/ADPase). Extracellular adenine nucleotides, for example, ATP and ADP, have been considered as mediators of inflammation [110]. This is based on the fact that adenine nucleotides trigger platelet aggregation, production of ROI by neutrophils and macrophages, and proliferation of mesangial cells. The administration of adenine nucleotide analogues (ATPγS, ADPβS) increases intraglomerular platelet aggregation and leukocyte production of superoxide in anti-Thy 1 glomerulonephritis [111]. In contrast, the administration of 2-chloro-adenosine inhibits intraglomerular platelet aggregation, leukocyte superoxide production, and proteinuria in the early phase of anti-Thy 1 glomerulonephritis [111]. These data indicate the proinflammatory activities of extracellular adenine nucleotides in the glomerulus. ATP and ADP are converted to adenosine by ATP/ADPase. ATP/ADPase is therefore potentially anti-inflammatory via the removal of proinflammatory ATP/ADP and via the generation of the anti-inflammatory molecule, adenosine.

In the glomerulus, ADPase activity is detected in the GBM [112]. The role of endogenous ADP/ATPase in glomerulonephritis has been extensively investigated by Bakker et al [110]. Depressed activity of glomerular ADPase is observed in experimental endotoxemia, anti-Thy 1 glomerulonephritis, and anti-GBM nephritis in which the procoagulant state is present [113, 114]. Poelstra et al reported that impairment of ADPase alone did not induce platelet aggregation in the glomerulus, but facilitated it under certain prothrombotic conditions [115]. Using *ex vivo* perfusion of isolated kidneys with platelets and ADP, they also showed that selective inhibition of ADPase resulted in enhanced platelet aggregation in the glomerulus [116]. These data show that endogenous ADP/ATPase is a component of glomerular self-defense against platelet-mediated injury.

Inactivators of leukocytes and resident cells

One of the most common pathological features of glomerular disease is infiltration of leukocytes. These are mainly monocytes/macrophages, with neutrophils and T lymphocytes present in smaller numbers [117]. Several investigators have disclosed a link between leukocyte infiltration and structural/functional alteration of the glomerulus [118]. Using experimental glomerulonephritis in combination with leukocyte depletion, the roles of leukocytes in glomerular injury have been established.

During resolution of acute glomerulonephritis, acti-

vated leukocytes must be deactivated. Several mediators, including peptide factors and bioactive lipids, have been postulated as "deactivators" of inflammatory leukocytes. Some of these are produced in normal glomeruli, and the production is up-regulated under pathologic situations. At inflammatory sites, the cooperation of leukocyte inactivators may play a role in the prevention of or recovery from glomerulonephritis.

After the initiation of glomerulonephritis, leukocytes and platelets activate resident cells via releasing pathogenic mediators. Once activated, however, resident cells may elaborate a set of "self-inactivators" and thereby regulate their activity. This autoregulation may be important to control inflammatory processes in the glomerulus. The distribution and putative function of these anti-inflammatory mediators in the glomerulus are addressed below.

(1) Anti-inflammatory cytokines.

(a) Transforming growth factor-β1 (TGF-β1). Transforming growth factor-β1 is a pleiotropic, "Janus-faced" cytokine involved in a wide range of pathophysiological processes. Overexpression of TGF-β1 is closely linked to certain pathologies, including fibrogenesis [31]. On the other hand, there are lines of evidence that support the bright side of TGF-β1. TGF-β1 is known to be a potent regulator of immune systems and inflammatory processes, generally functioning as an endogenous anti-inflammatory molecule [119, 120]. TGF-β1 exerts anti-inflammatory actions in several diseases, especially in autoimmune disorders (abstract; Pankewycz et al, *J Am Soc Nephrol* 5:761, 1994) [121–124]. Constitutive expression of TGF-β1 is essential for the maintenance of normal immune and organ functions, as targeted disruption of TGF-β1 via homologous recombination induced immune dysregulation, multiorgan inflammation, and early death in mice [125–127].

Normal glomeruli express or contain detectable levels of TGF-β1 [38, 128–130]. The physiological meaning of this observation is currently unknown. Based on the fact that TGF-β generally functions as a differentiation factor, the basal levels of TGF-β1 could contribute to the maintenance of normal glomerular structure and function. In various pathological conditions, TGF-β1 is up-regulated in the glomerulus. The induction of TGF-β1 has been reported in experimental models, including anti-Thy 1 glomerulonephritis, anti-GBM nephritis, Habu-venom glomerulonephritis, PAN nephrosis, and diabetic glomerulopathy, as well as in human diseases such as IgA nephropathy, focal/segmental glomerulosclerosis, crescentic glomerulonephritis, lupus nephritis, diabetic nephropathy, and human immunodeficiency virus nephropathy [31, 130]. The cell type responsible for TGF-β1 production in the glomerulus is not fully elucidated, but mesangial cells may be one of the major sources [38, 131]. Cultured glomerular epithelial cells also produce TGF-β1 [132]. *In vitro*, mesangial cells have the ability to secrete latent TGF-β1 abundantly and to convert it to the mature form [133–135]. The production of TGF-β1 by mesangial cells and epithelial cells may be enhanced by a variety of factors, including angiotensin II, atrial natriuretic peptide, thrombin, thromboxane, high glucose, oxidized lipoprotein, immune complexes, and mechanical stress [132, 136–143]. In pathologic circumstances, TGF-β1, TGF-β2, and TGF-β3 may be similarly up-regulated in the glomerulus [130], but the pathophysiological role and kinetics of TGF-β2 and TGF-β3 remain to be disclosed.

In the generation of glomerulonephritis, macrophage-derived proinflammatory cytokines play crucial roles. Previous reports provided evidence that TGF-β1 inhibits production of IL-1α, TNF-α, lymphotoxin, and interferon-γ by peripheral blood mononuclear cells and peritoneal macrophages [144, 145]. We recently showed that cultured mesangial cells secrete a factor that strongly inhibits production of IL-1β, IL-6, TNF-α, and monocyte chemoattractant protein-1 (MCP-1) by activated macrophages. We have identified that this active entity is TGF-β1 [134, 146].

Reactive oxygen/nitrogen species play a role in a wide range of glomerular injury. In glomerulonephritis, major sources of ROI and NO are neutrophils and monocytes/macrophages [147, 148]. It is known that TGF-β1 inhibits production of ROI and NO by macrophages at picomolar concentrations [149, 150]. Locally produced TGF-β1, possibly, functions as a safeguard molecule that attenuates ROI/NO-mediated, glomerular injury.

TGF-β1 is an inhibitor of mitogenesis in cultured glomerular cells. Several investigators have shown that, *in vitro*, externally added TGF-β1 inhibits mitogenesis of cultured mesangial, epithelial, and endothelial cells [151–153]. Similarly, the proliferative response of isolated glomeruli is inhibited by externally added TGF-β1 [154]. Using an *ex vivo* gene transfer approach [155], we have demonstrated that mitogenic responses of glomerular cells in acute anti-Thy 1 glomerulonephritis could be repressed by the introduction of a gene coding for the active form of TGF-β1 [154].

In certain cell types, TGF-β counteracts the effects of proinflammatory cytokines [156–158]. Previous reports showed that mesangial cells, endothelial cells, and isolated glomeruli treated with TGF-β1 exhibited depressed responses to IL-1β and TNF-α (abstract; Danoff and Jiang, *J Am Soc Nephrol* 7:1696, 1996) [154, 159, 160]. Mesangial cells stably transfected with a cDNA encoding the active form of TGF-β1 exhibit blunted expression of stromelysin in response to the macrophage cytokine IL-1β [1]. In contrast, mesangial cells transfected with a dominant-negative mutant of TGF-β type II receptor show enhanced responses to IL-1 [1]. This result indicates that TGF-β functions as an autocrine inhibitor of

the cytokine response in mesangial cells. In isolated rat glomeruli, externally added TGF-β1 repressed the induction of stromelysin by macrophage-derived cytokines. Similarly, when isolated nephritic glomeruli producing active TGF-β1 (anti-Thy 1 nephritis, day 7) were stimulated by IL-1β, the induction of stromelysin was depressed, compared with normal glomeruli [1].

We previously reported that *in vivo* transfer of activated macrophages induces the expression of stromelysin in resident glomerular cells [161]. To investigate whether endogenous TGF-β1 suppresses the macrophage-triggered glomerular cell activation, LPS-stimulated reporter macrophages were transferred into normal rat glomeruli, and glomeruli were subjected to anti-Thy 1 glomerulonephritis (day 7). Compared with normal glomeruli, the nephritic glomeruli secreted higher levels of biologically active TGF-β. As described earlier in this article, in the normal glomeruli, stromelysin mRNA was markedly induced in resident cells after the transfer of activated macrophages. This induction was dramatically suppressed in the nephritic glomeruli producing active TGF-β1 [1]. Similar suppression was also observed in the induction of other cytokine-inducible molecules, including gelatinase B [2] and inducible NO synthase (iNOS) (abstract; Sütö and Kitamura, ibid). Based on this evidence together with the *in vitro* data, TGF-β1 can be regarded as a potential defender molecule that attenuates activation of resident cells faced by inflammatory macrophages.

(b) Interleukin-4. Interleukin (IL)-4 is a cytokine that is produced mainly by T-cell helper type 2 (Th2). It has been shown that IL-4–positive T cells are present in glomeruli of a rat model of anti-GBM nephritis and human glomerulonephritis, including IgA nephropathy and lupus nephritis [162, 163]. However, IL-4 may also be produced by resident glomerular cells. Using *in situ* hybridization, Furusu et al reported that IL-4 mRNA is induced in mesangial cells and podocytes in human glomerulonephritis, including IgA nephropathy, lupus nephritis, membranous nephropathy, and minimal change nephrotic syndrome [164].

Interleukin-4 is a potent inactivator of macrophages. IL-4 inhibits production of IL-1β, IL-8, and MIP-1α, stimulates the expression of IL-1 receptor antagonist and IL-1 decoy receptor, and suppresses the production of ROI and NO in macrophages and neutrophils [165]. Tipping et al reported that the administration of IL-4 attenuated crescent formation, accumulation of T cells/macrophages and proteinuria in a murine model of anti-GBM nephritis [166]. Using IL-4 null mutant mice, Kitching et al recently showed that IL-4 deficiency amplified local and systemic Th1 responses and exacerbated anti-GBM glomerulonephritis [167]. Although there is some controversy [168, 169], locally produced IL-4 may participate in the attenuation of glomerular inflammation.

In addition to its effects on leukocytes, IL-4 modulates the function of resident glomerular cells. For example, IL-4 antagonizes the effect of IL-1 and inhibits proliferation of mesangial cells [170]. Cytokine induction of E-selectin in glomerular endothelial cells is also inhibited by IL-4 [171]. Endogenous IL-4 may attenuate inflammatory processes via inhibiting accumulation of leukocytes and cytokine responses of resident cells.

(c) Interleukin-6. Interleukin-6 is a multifunctional cytokine produced by various cell types and regulates growth and differentiation of a wide range of cells. Cultured glomerular epithelial and mesangial cells have the ability to produce IL-6, and the production is increased by inflammatory stimuli, including LPS, IL-1β, TNF-α, granulocyte inhibitory protein, angiotensin II, and immune complexes [172–177]. Expression of IL-6 may be detectable in the mesangium of normal human glomeruli [178]. The mesangial expression of IL-6 is markedly up-regulated during glomerular inflammation, especially mesangial proliferative glomerulonephritis [173, 178, 179].

Initially, IL-6 was considered to be a proinflammatory cytokine because IL-6 is an activator of lymphocytes and induces proliferation and MCP-1 expression by mesangial cells *in vitro* and *in vivo* [173, 180, 181]. Systemic administration with IL-6 exacerbated, but blockade of IL-6 receptor ameliorated murine lupus nephritis [182, 183], indicating a pathogenic role of IL-6 in certain types of glomerulonephritis. However, subsequent investigation pointed that this story is not simple. Some investigators reported that IL-6 did not induce or rather inhibited proliferation of mesangial cells *in vitro* and *in vivo* [184–187]. Rees et al reported that administration with IL-6 attenuated glomerular thrombosis, leukocyte infiltration/activation, expression of cytokines/chemokines, and proteinuria in nephrotoxic serum nephritis (abstract; Karkar et al, *J Am Soc Nephrol* 6:834, 1995) [11, 188]. The putative, anti-inflammatory properties of IL-6 are as follows: (*a*) up-regulation of circulating cortisol concentrations and (*b*) suppression of cytokine production (IL-1β, TNF-α, IL-8, MCP-1), induction of cytokine inhibitors (IL-1ra, IL-1 decoy receptor, soluble IL-1 receptor, sTNFR), and inhibition of ROI/NO production by macrophages [165]. Using IL-6 null mutant mice, a recent investigation clearly showed that IL-6 is an anti-inflammatory cytokine required for controlling local and systemic inflammatory responses [189]. In certain pathologic circumstances, endogenous IL-6 synthesized by resident cells may be involved in the glomerular self-defense against inflammation.

(d) Interleukin-10. Interleukin-10 is a multifunctional cytokine that is an important regulator of lymphoid and myeloid cell functions. IL-10 is produced mainly by Th2 cells and has profound effects on the morphology, phenotype, and production of bioactive substances by monocytes/macrophages. IL-10 inhibits macrophage produc-

tion of reactive oxygen/nitrogen species and cytokines, including IL-1α, IL-1β, IL-8, TNF-α, granulocyte macrophage colony-stimulating factor (GM-CSF), and granulocyte colony stimulating factor (G-CSF) [190–192]. IL-10 enhances the synthesis of cytokine inhibitors, IL-1ra, and sTNFR [193, 194]. Several investigators reported the utility of IL-10 for the treatment of experimental glomerulonephritis. Tipping et al showed that, like IL-4, pretreatment with IL-10 attenuated crescent formation, accumulation of T cells/macrophages, proteinuria, and renal insufficiency in a murine model of anti-GBM nephritis [166]. Kitching et al showed that systemic administration of IL-10 in combination with IL-4 reduced the severity of anti-GBM nephritis in mice even after the establishment of the disease [195].

Interleukin-10 is produced in nephritic glomeruli [162]. The cells responsible for the production may be inflammatory leukocytes, but activated mesangial cells also produce IL-10 *in vitro* [196, 197]. Fouqueray et al reported a possibility that IL-10 functions as an autocrine inhibitor of cytokine production in mesangial cells [197]. Interestingly, they also observed that the production of IL-10 by mesangial cells was stimulated by the anti-inflammatory cytokine, TGF-β. In contrast, Chadban et al recently reported that IL-10 may act as an autocrine stimulator of mesangial cell proliferation both *in vitro* and *in vivo* [196]. Further investigation will be required to determine the role for IL-10 in the regulation of glomerular cell activity.

(e) Interleukin-13. Interleukin-13 is an IL-4–like Th2 cytokine that modulates the function of monocytes/macrophages and B cells. IL-13 inhibits production of various proinflammatory cytokines (IL-1α, IL-1β, TNF-α), chemokines (IL-8, MIP-1α), and leukocyte growth factors (GM-CSF, G-CSF) by monocytes/macrophages [198]. IL-13 also inhibits production of NO by monocytes/macrophages and increases their expression of IL-1ra [198]. It has been shown that the expression of IL-13 mRNA was detectable in kidneys subjected to anti-GBM glomerulonephritis [199]. Currently, it is unclear whether resident glomerular cells produce IL-13.

(f) Leukemia-inhibitory factor (LIF). Leukemia-inhibitory factor is a pleiotropic cytokine produced by a wide range of cell types. Low levels of LIF expression are detectable in normal rat glomeruli [200]. Up-regulation of LIF is observed in nephritic glomeruli, including anti-GBM nephritis [200]. The role of LIF in the pathogenesis of glomerular inflammation is currently unclear, but Tang et al reported that systemic administration of recombinant LIF ameliorated experimental anti-GBM nephritis. That is, it reduced macrophage infiltration, local expression of cytokines, and proteinuria [200]. Cultured mesangial cells produce low levels of LIF. The transcriptional expression and protein synthesis are dramatically up-regulated in response to inflammatory stimuli, including IL-1β, TNF-α, PDGF, and LPS [201]. Under certain pathological milieus, mesangial cell-derived LIF may function as a defending molecule against inflammation.

(2) Anti-inflammatory eicosanoids.

Eicosanoids serve as important modulators for various pathophysiological processes in the glomerulus. Eicosanoids affect the function of leukocytes and resident cells and participate in the generation of glomerular disease, especially glomerulonephritis [202]. A majority of eicosanoids, including thromboxane A2, prostaglandin (PG) $F_{2\alpha}$, leukotrienes B_4, C_4, and D_4, and 15-, 12- and 5-hydroxyeicosatetraenoic acids are regarded to be proinflammatory. These mediators trigger leukocyte infiltration, coagulation and proliferation and matrix production by resident cells [202]. On the other hand, some eicosanoids function as anti-inflammatory factors. These include prostacyclins (PGE_2, PGI_2) and lipoxins (LXA_4, LXB_4) [202, 203].

(a) Prostacyclins (PGE_2, PGI_2). Prostacyclins are powerful autacoids with potential effects on the immune system. Among these, $PGF_{2\alpha}$ acts as a proinflammatory mediator because it facilitates neutrophil chemotaxis [204]. In contrast, PGE_2 and PGI_2 are anti-inflammatory. PGE_2 suppresses functions of lymphocytes, natural killer cells, macrophages, and neutrophils [205–209]. Importantly, PGE_2 and PGI_2 inhibit neutrophil chemotaxis [210] and production of IL-1β and TNF-α by activated macrophages [209, 211]. In addition, PGI_2 inhibits platelet aggregation [212].

Prostacyclins modulate not only leukocyte/platelet function but also the activity of resident glomerular cells. For example, $PGF_{2\alpha}$ acts as a mitogen for cultured mesangial cells [213]. In contrast, PGI_2 inhibits mesangial cell proliferation [213], and PGE_2 represses production of type I and III collagens by mesangial cells [214].

It has been shown that cultured glomerular endothelial, epithelial, and mesangial cells have the ability to produce PGE_2 and PGI_2 [215–217]. Normal glomeruli produce PGE_2, which is derived mainly from mesangial cells [218]. *In vitro*, the production of PGE_2 by mesangial cells is up-regulated by a variety of inflammatory mediators, including IL-1β, TNF-α, IL-6, PDGF, angiotensin II, arginine vasopressin (AVP), endothelin-1, thrombin, ROI, NO, and immunoglobulins [219–225]. The production of PGE_2 and PGI_2 by glomerular epithelial cells is increased by AVP [226]. *In vivo*, enhanced production of PGE_2 is observed in anti-GBM nephritis and passive Heymann nephritis [227, 228]. Based on the anti-inflammatory properties of PGE_2 and PGI_2, these molecules may function as endogenous defenders against glomerular inflammation. Indeed, McLeish et al reported that systemic administration of PGE_2 resulted in attenuation of hypercellularity, immunoglobulin deposition, and proteinuria, as well as preservation of renal function in immune complex glomerulonephritis in mice [229, 230]. Poelstra et al reported that, in anti-Thy 1 glomerulone-

phritis, the PGI_2 analogue Iloprost inhibited intraglomerular platelet activation and reduced proteinuria [231].

(b) Lipoxins (LXA_4, LXB_4). Lipoxin is a family of eicosanoids with potential anti-inflammatory properties. Lipoxins counteract the pathogenic action of leukotrienes produced in various glomerular diseases [202, 203]. For example, LXA_4 and LXB_4 inhibit leukotriene B_4-mediated neutrophil chemotaxis, adhesion to endothelial cells, and transmigration across the vascular wall [232, 233].

Increased production of LXA_4 is observed in certain glomerular diseases, including anti-GBM nephritis and concanavalin A-ferritin glomerulonephritis [234, 235]. Neutrophil–platelet interaction is considered to be the major pathway for the lipoxin formation [203]. That is, platelet lipoxygenase (12-LO) converts the neutrophil-derived leukotriene A_4 to lipoxins. However, Garrick et al reported that rat mesangial cells also have the ability to generate LXA_4 from leukotriene A_4 [236]. A subsequent study showed that 12- and 15-LO activity is present in mesangial cells and is responsible for the production of LXA_4 and LXB_4 [237]. Katoh et al reported that glomerular 12/15-LO mRNA is up-regulated in rat glomeruli with nephrotoxic serum nephritis [238]. Interestingly, this up-regulation was correlated with increased expression of IL-4, a well-known trigger for LO expression. *In vitro*, externally added LXA_4 and LXB_4 cooperatively inhibited adhesion of neutrophils to mesangial cells [239]. *Ex vivo* exposure of rat neutrophils to LXA_4 attenuated their recruitment to inflamed glomeruli following the induction of concanavalin A-ferritin glomerulonephritis [234]. Resident cell-derived lipoxins may play a regulatory role in limiting the accumulation of neutrophils during glomerular disease.

Leukotriene D_4 is potentially proinflammatory and mitogenic to cultured mesangial cells [240]. It has been shown that LXA_4 antagonizes the action of leukotriene D_4 on mesangial cells. This might be attributed to competition of LXA_4 with leukotriene D_4 for binding to cell surface [241].

(3) Apoptotic cells.

Recently, Voll et al reported the possibility that apoptotic cells negatively regulate the function of monocytes/macrophages [242]. Monocytes incubated with apoptotic cells showed increased secretion of the anti-inflammatory cytokine IL-10 and decreased production of proinflammatory cytokines IL-1, IL-12, and TNF-α. This anti-inflammatory action of apoptotic cells was consistently observed, regardless of cell types or triggers for apoptosis. Fadok et al reported that phagocytosis of apoptotic neutrophils suppressed production of IL-1β, IL-8, GM-CSF, TNF-α, leukotriene C_4, and thromboxane B_2 by activated macrophages [243]. In contrast, the production of anti-inflammatory mediators TGF-β1 and PGE_2 was up-regulated in the presence of apoptotic cells. In the glomerulus, apoptosis of glomerular cells, especially mesangial cells, is observed in various glomerular diseases [244–248]. Apoptotic mesangial cells could play a role in subsidence of inflammatory macrophages in acute glomerulonephritis.

(4) Mesangial matrix.

The mesangial matrix is a structural constituent that supports glomerular architecture. Recent investigation disclosed a possibility that the mesangial matrix may serve as an safety device that halts mesangial cell activation and dedifferentiation. Several lines of evidence supports this hypothesis. (*a*) Within three-dimensional ECM reconstituted *in vitro*, mesangial cells exhibit little mitogenic activity and lower levels of α-smooth muscle actin (α-SMA) expression compared with the cells on plastic [249–251]. (*b*) Cultured mesangial cells incorporated in "hillocks," the three-dimensional structures of natural ECM created by mesangial cells, show depressed mitogenic activity and attenuated α-SMA expression [251, 252]. (*c*) Following the destruction of the mesangial matrix by Habu venom, mesangial cells exhibit remarkable mitogenesis, which is gradually attenuated in parallel with the reconstruction of the mesangial matrix [253]. (*d*) Overexpression of matrix-degrading proteinases is observed in experimental and human glomerulonephritis, and it is associated with mesangial cell activation *in vivo* (abstract; Miyazaki et al, ibid) [41–45]. (*e*) Enhancement of matrix-degrading activity via transfection with a certain MMP gene or external supply with active MMP protein induces accelerated mitogenesis of cultured mesangial cells [49, 51]. (*f*) Inhibition of endogenous matrix-degrading activity via either antisense RNA, ribozyme, or a pharmacological inhibitor results in the suppression of mitogenic activity and α-SMA expression in cultured mesangial cells [49, 50]. (*g*) Pharmacological inhibition of MMP *in vivo* attenuates mesangial cell proliferation in anti-Thy 1 glomerulonephritis [254]. These data suggest a role for the mesangial matrix in the suppression of mesangial cell activation.

The mechanisms involved in the regulatory effect of three-dimensional ECM on mesangial cell phenotype are not yet determined, but recent investigations have raised some possibilities. Marx et al found that three-dimensional cultures using a collagen gel resulted in a remarkable down-regulation of PDGF β receptors on the surface of mesangial cells [255]. We have reported that an ECM-initiated signal(s) suppresses activation of mesangial cells via inactivation of CArG box element, the *cis*-element required for mitogenesis and α-SMA expression [251].

Inhibitors of platelet function and thrombogenesis

Pathogenic roles of platelets in glomerular injury have been well documented. In addition to their contribution to thrombogenesis, platelet is an important source of various, potentially pathogenic mediators. These include

eicosanoids, growth factors, vasoactive amines, proteolytic enzymes, ROI, complement, adenine nucleotides, and so on [256]. The release of these molecules from activated platelets subsequently induces glomerular cell proliferation and ECM synthesis, facilitates leukocyte influx and immune complex deposition, and alters glomerular hemodynamics, leading to glomerular injury [256]. In this section, we address endogenous, glomerular machinery that attenuates platelet activation and thrombogenesis.

(1) Thrombomodulin.

Thrombomodulin is a glycoprotein found on endothelial cells. Thrombomodulin acts as a cofactor for the thrombin-catabolized activation of protein C and inhibits thrombogenesis. Thrombin binding to thrombomodulin induces internalization of the complex with transport to lysosomes, leading to its degradation. This suggests that thrombomodulin functions as a "scavenger receptor" for thrombin. In the normal glomerulus, a small amount of thrombomodulin is present along the endothelium [257]. In membranoproliferative glomerulonephritis and lupus nephritis, the amount of endothelial thrombomodulin is increased [258]. In contrast, *in vitro*, thrombomodulin activity in normal glomeruli is repressed by inflammatory stimuli including TNF-α and LPS [259]. A recent report showed that, in addition to endothelial cells, mesangial cells have the ability to produce thrombomodulin both *in vitro* and *in vivo* [260]. Systemic administration of recombinant thrombomodulin inhibited glomerular fibrin deposition in experimental endotoxemia in rats [261], suggesting that locally produced thrombomodulin may serve as a defending molecule with anticoagulant activity.

(2) Tissue factor pathway inhibitor (TFPI).

Tissue factor pathway inhibitor is a factor Xa-dependent feedback inhibitor of the tissue factor-factor VIIa complex. Systemic administration of a two-domain TFPI analogue significantly reduced endotoxin-induced, massive fibrin deposition in the glomerulus [262]. Normally, TFPI is produced by glomerular cells [263]. Human mesangial cells synthesize TFPI *in vitro*, and its production is up-regulated by heparin [264]. Erlich et al provided evidence that TFPI is an endogenous defender that counteracts fibrinogenesis in certain glomerular disease [263]. They showed the following: (*a*) Glomerular TFPI synthesis was initially decreased in fibrin-dependent, crescentic glomerulonephritis. (*b*) *In vivo* inhibition of TFPI using an anti-TFPI antibody exacerbated glomerular fibrin deposition and renal impairment. (*c*) Infusion of recombinant human TFPI significantly reduced glomerular fibrin deposition, proteinuria, and renal impairment in this nephritis model.

(3) Plasminogen activators.

Thrombi, initially composed of aggregated platelets, are gradually replaced by fibrin. The fibrin is then digested by fibrinolytic enzymes released from endothelial cells and other cell types. The fibrinolysis is mediated by conversion of a plasminogen to the active proteolytic enzyme, plasmin, via the tissue-type (t-PA) and urokinase-type (u-PA) plasminogen activators. Fibrin deposition is a characteristic feature of certain experimental and human glomerulonephritis. For example, in anti-GBM nephritis, fibrinolytic activity is reduced in parallel with fibrin deposition [265]. This is mainly due to decreased expression of t-PA and increased plasminogen activator inhibitor-I. It has been shown that the systemic administration of t-PA protected animals from glomerular fibrin deposition, crescent formation, and renal dysfunction in anti-GBM nephritis [266].

Isolated human glomeruli produce both t-PA and u-PA [267]. Immunohistochemical analysis showed that t-PA is present exclusively in the endothelium, but u-PA exists in the cytoplasm of glomerular epithelial cells [267]. The amount of t-PA is increased in certain human glomerular diseases, including IgA nephropathy and lupus nephritis [268]. In contrast, the amount of u-PA may be decreased in glomeruli of lupus-prone mice [269]. *In vitro*, mesangial cells produce t-PA, but not u-PA [270]. In contrast, glomerular epithelial cells in culture synthesize abundant u-PA and small amount of t-PA [271]. The production of these PAs is up-regulated by thrombin, IL-1, and TNF-α [271, 272].

Recently, the crucial role of local t-PA in the attenuation of glomerular inflammation was demonstrated using gene knockout mice [273]. In this study, crescentic glomerulonephritis was induced in mice in which the genes for PAs had been disrupted by homologous recombination. They found that the deficiency of t-PA exacerbated the glomerular injury. In contrast, the deficiency of u-PA did not significantly affect severity of the disease. These data imply that t-PA is the major PA that protects the glomerulus from crescentic glomerulonephritis.

Other factors

(1) Heparin and heparan sulfate proteoglycan (HSPG)

Heparin is generally described as an anionic polysaccharide or a sulfated glycosaminoglycan with irregular sequences. Heparin has been considered a therapeutic agent for various glomerulonephrites. Systemic administration of heparin attenuates experimental and human glomerular diseases, including anti-GBM nephritis, Habu venom-induced injury, PAN nephrosis, remnant-kidney models, anti-Thy 1 glomerulonephritis, and lupus nephritis [274, 275]. The therapeutic effect of heparin is ascribed to its anti-inflammatory properties, that is, inhibition of coagulation/thrombosis, complement inactivation, suppression of leukocyte function, and inactivation of resident cells.

Antithrombin III inhibits the function of thrombin, factor Xa and factor IXa. This inhibition is markedly

enhanced by heparin and naturally occurring HSPG. HSPG is present on the luminal surface of endothelial cells and is the natural catalyst of the inhibitory effect of antithrombin III [276]. Heparin also inhibits platelet activation by neutrophils [277] and enhances endothelial production of NO, the potential suppressor of platelet aggregation and adhesion [278]. Additionally, heparin promotes fibrinolytic processes [279].

Heparin has the ability to inhibit complement activation *in vivo* [280, 281]. The inhibitory action is at multiple levels. Heparin inhibits C1 hemolytic function via interaction with and potentiation of C1 inhibitor [282]. C3 and C4 conversions by classical pathway activators are also inhibited by heparin [282]. Surface-associated heparin inhibits zymosan-induced activation of the alternative pathway by augmenting the action of complement regulatory proteins on particle-bound C3b [283].

Heparin has the potential to suppress the activity of pathogenic proteinases. For example, in vascular smooth muscle cells (SMCs), heparin inhibits phorbol ester-triggered induction of interstitial collagenase, gelatinase B, and stromelysin mRNAs, as well as the synthesis of these proteins [284]. We reported that, in mesangial cells, heparin selectively suppressed cytokine-mediated induction of stromelysin without affecting the expression of procollagen α1 (IV), laminin B2, and TIMP [55]. Heparin also inhibits the activity of neutrophil-derived enzymes, including elastase and cathepsin G [285, 286], both of which are potentially pathogenic to the glomerulus [46].

Heparin and HSPG inhibit leukocyte rolling and adhesion on the endothelium [286, 287]. This is, in part, due to (*i*) the inhibitory effect of heparin on the function of L- and P-selectin, the endothelial adhesion receptors required for capture and rolling of neutrophils [288] and (*ii*) the down-regulation of the adhesion receptor CD11b on the surface of neutrophils [289]. *In vitro*, heparin inhibits both random migration and directed locomotion of neutrophils toward zymosan-activated serum [290]. *In vivo*, the intravenous administration of heparin diminishes the influx of neutrophils into the peritoneal cavity of thioglycollate-treated mice [288]. Heparin inhibits cellular aggregation, degranulation, and superoxide anion generation by activated neutrophils [291]. Heparin inhibits cytotoxicity exerted by neutrophils [292] and may induce their apoptosis [293]. Additionally, heparin also attenuates certain function of lymphocytes and macrophages [281, 294].

Heparin and HSPG have direct actions on resident glomerular cells, especially mesangial cells. Heparin and HSPG inhibit mitogenesis, migration, and expression of immediate early genes (c-*fos* and *egr-1*) in cultured mesangial cells [295–300] and increase their production of HSPG [301]. Recently, we found that heparin inhibits spontaneous apoptosis of podocytes in isolated rat glomeruli and oxidant-induced apoptosis in cultured mesangial cells (abstract; Ishikawa and Kitamura, *J Am Soc Nephrol* in press) [302]. Heparin and HSPG may also alter the function of glomerular epithelial and endothelial cells. These molecules inhibit proliferation of glomerular epithelial cells and suppress their responses to EGF [303, 304]. Heparin facilitates release of EC-SOD from endothelial cells, leading to their protection from ROI-mediated injury [305, 306].

Heparin-like species are widely distributed in the glomerulus, especially in GBM and the mesangial matrix [307, 308]. The amount of HSPG in the glomerulus is increased in experimental glomerular diseases, including anti-Thy 1 glomerulonephritis, PAN nephrosis, and renal ablation models [309]. *In vitro*, glomerular cells produce heparin-like glycosaminoglycans. For example, glomerular epithelial and endothelial cells secrete heparin-like molecules, which suppress mesangial cell proliferation [295, 296]. Mesangial cells and epithelial cells also produce HSPG, which functions as an autocrine or paracrine growth inhibitor [297, 304, 310, 311]. Interestingly, angiotensin II, an well-recognized pathogenic mediator for glomerular disease, suppresses production of HSPG, but TGF-β1, a putative defending molecule against acute glomerular injury, stimulates the production of HSPG in mesangial cells [312, 313]. The intercellular network via heparin-like molecules may be an important defense mechanism that protects resident cells from activation and injury.

(2) Nitric oxide (NO).

The L-arginine-NO pathway is involved in the physiological function of mammalian organs, including the kidney. In the glomerulus, NO synthase is constitutively expressed, and the basal generation of NO participates in the regulation of glomerular hemodynamics [314].

Locally produced NO may function as an oxygen radical scavenger in the glomerulus. In the presence of NO, superoxide produced by activated cells is rapidly combined with NO. This reaction results in superoxide scavenging, leading to the mitigation of its cytotoxic effect [315]. Glomerular cells, especially mesangial cells, have the ability to express iNOS and synthesize NO in response to proinflammatory stimuli, including IL-1β and TNF-α [316, 317]. Sandau, Pfeilschifter and Brune reported that exogenously supplied superoxide induced apoptosis of mesangial cells and that NO inhibited the cytotoxic effect of superoxide anion [318].

Nitric oxide is an endogenous inhibitor of leukocyte function, acting particularly on neutrophils. Kubes, Suzuki and Granger reported that suppression of endothelial production of NO facilitated adhesion of neutrophils to the endothelium via up-regulation of CD11/CD18 expression [319]. NO also functions as an inhibitor of neutrophil superoxide production via direct action on the NADP oxidase [320]. It has been shown that NO released

by resident cells protects the cells from neutrophil-induced cytotoxicity [321]. These results suggest that endothelium-derived NO is an important endogenous inhibitor of leukocyte adherence and cytotoxic action.

Nitric oxide inhibits platelet adhesion to the damaged endothelium and platelet aggregation [322, 323]. Shultz and Raij showed that endogenous NO prevents endotoxin-induced glomerular thrombosis in rats [324]. This was further supported by Westberg et al, who showed that the administration of NO inhibitor deteriorated, but NO donor prevented glomerular thrombosis in a rodent model of endotoxemia [325].

Overproduction of NO in the glomerulus is supposed to be proinflammatory via induction of glomerular cell injury [326]. However, subtoxic levels of NO exert some beneficial actions on resident cells. For example, externally supplied NO suppresses proliferation, ECM production, and expression of ICAM-1 in cultured mesangial cells [139, 327–329]. NO also suppresses oxidant-initiated apoptosis of mesangial cells via scavenging superoxide anion [318]. NO may inhibit leukocyte–endothelial interaction via down-regulation of adhesion receptors on the surface of endothelial cells [330]. Recently, Haberstroh et al showed that endogenous NO suppresses LPS-triggered expression of Rantes in the glomerulus [331].

In vitro, the L-arginine-NO pathway is constitutively active in glomerular endothelial cells. The activity may be up-regulated by inflammatory mediators, including bradykinin, extracellular 5′ adenosine triphosphate (ATP), thrombin, and platelet-activating factor [148]. In cultured mesangial cells, the L-arginine-NO pathway is inducible in response to proinflammatory stimuli, including LPS, IL-1, TNF-α, and interferon-γ [148]. In certain pathologic circumstances, endogenous NO may function as a part of the self-defense program in the glomerulus.

Intracellular defense

Heat shock proteins

Heat shock protein is a family of highly conserved proteins synthesized under various pathophysiological circumstances. These molecules are classified into five major subfamilies: 110 kDa HSP (HSP110), 90 kDa HSP (HSP90), 70 kDa HSP (HSP70), 60 kDa HSP (HSP60), and low-molecular weight HSP [332]. Generally, constitutively expressed HSPs control maturation and turnover of intracellular proteins and thereby play a role in the maintenance of cellular integrity. On the other hand, inducible HSPs are synthesized in response to environmental perturbations, including ischemia, inflammation, oxidative stress, and toxin exposure and function as defending molecules that protect tissues from further injuries [332]. Recent investigations have disclosed that several HSPs are expressed in normal kidneys and that their expression pattern and levels are altered after pathologic insults [333].

(1) HSP70 family.

The HSP70 family includes two major members: a 73 kDa protein that is constitutively expressed and a 72 kDa protein that is inducible in response to stresses [332]. Constitutive HSP73 participates in intracellular protein targeting, processing and transport, and thereby plays an essential role in the maintenance of cell function. In response to a wide range of stress, HSP72 is rapidly synthesized and acts as a molecular chaperone; that is, with HSP60, this molecule binds to denatured or unfolded proteins and subsequently renatures or refolds the damaged proteins [334, 335]. This allows the cells to be protected against further injury and facilitates their recovery from established damage.

In normal rat glomeruli, HSP70 is expressed in podocytes and Bowman's epithelium [336]. Glomerular expression of HSP70 is up-regulated in mesangial cells in PAN nephrosis [336]. Currently, the expression and kinetics of HSP70 in human glomerular diseases remain to be elucidated. *In vitro*, cultured mesangial cells constitutively express HSP70 [337]. Mesangial cells transfected with a HSP70 cDNA acquire resistance to oxidant-induced apoptosis [337]. This protective effect might be due, in part, to the ability of HSP70 to suppress activation of c-Jun N-terminal kinase (JNK) [338], a putative mediator for oxidant-induced apoptosis in mesangial cells [339].

In certain pathological circumstances, the constitutive expression of HSP70 in the glomerulus is down-regulated. We found that glomerular expression of HSP70 was suppressed in rat kidneys subjected to anti-GBM glomerulonephritis. The down-regulation of HSP70 was caused, at least in part, by IL-1 and was associated with the incidence of glomerular cell apoptosis *in vivo* [337]. Similarly, mesangial cells treated with IL-1β showed depression of HSP70 and an increase in susceptibility to oxidant-triggered apoptosis. Transfection of mesangial cells with a HSP70 cDNA overcame the apoptosis-priming effect of IL-1β [337]. Depression of HSP70 by inflammatory cytokines may magnify glomerular injury.

(2) HSP60 and HSP90 families.

The HSP60 family of molecules acts as a molecular chaperone that facilitates proper maturation of newly synthesized proteins [334, 335]. The members of this family facilitate folding of monomeric proteins and catalyze the higher ordered assembly of oligomeric complexes [332]. HSP60 is constitutively expressed in mammalian kidneys [340], but its intrarenal distribution is not determined.

HSP90 is an abundant cytosolic protein, and its expression is up-regulated by certain stresses. This molecule is now regarded as a chaperone involved in signal transduction. HSP90 interacts with a large number of other cellular proteins, including tyrosine kinases and steroid receptors and thereby regulates the kinetics and activity of these signaling proteins [335]. In the normal glomerulus,

HSP90 is present in podocytes [341]. This constitutive expression may be involved in physiological functions of the cells via interacting with particular target proteins.

Currently, the contribution of HSP60 and HSP90 to glomerular self-defense is unknown. Recent reports suggested the possibility that these molecules could be involved not in the prevention, but in the generation of diseases. For example, HSP60 may serve as an antigenic determinant to which cytotoxic T cells respond [342]. In certain cell types, increased HSP90 may rather enhance cellular damage [343]. Further investigation is needed to disclose the distribution, kinetics, and pathophysiological roles of these HSPs in the glomerulus.

(3) HSP27.

Like HSP70, HSP27 participates in cellular defense. Overexpression of HSP27 leads to reinforcement of cellular resistance to stresses, including heat shock and ROI [332]. In human kidneys, the expression of HSP27 is observed predominantly in collecting ducts and modestly in proximal/distal tubules and mesangial cells [344].

In the rat, HSP27 is localized in glomerular epithelial cells [345]. HSP27 may be involved in physiological function of podocytes. In PAN nephrosis, the effacement of foot processes is closely associated with the induction and phosphorylation of HSP27 [345]. The structure of foot process—an essential part of the filtration barrier—is controlled by actin microfilaments, and actin polymerization is known to be regulated by HSP27. The altered activity of HSP27 in podocytes might therefore cause aberrant foot process structure, leading to the development of proteinuria.

(4) HSP32 (heme oxygenase-1).

Heme oxygenases are microsomal enzymes that catalyze the rate-limiting step in the degradation of heme to biliverdin, free ion, and carbon monoxide. Two isoforms of heme oxygenase have been identified. Heme oxygenase-1 is the stress-inducible form that is also termed HSP32, and heme oxygenase-2 is the constitutive isoform that is present under physiological conditions [346]. Various environmental factors trigger expression of heme oxygenase-1. These include heat shock, heme compounds, heavy metal ions, cytokines, and oxidative stress [346]. In particular, the induction of heme oxygenase-1 is regarded as a general response to oxidant stress in mammalian cells [347] and is implicated in a defense mechanism against ROI [348]. Indeed, transfection with a heme oxygenase-1 cDNA confers resistance of epithelial cells to oxidant stress [349].

Heme oxygenase-1 is induced under inflammatory situations. The role of heme oxygenase-1 in the inflammatory milieu is not fully determined, but recent investigations showed that heme oxygenase-1 attenuates inflammatory responses [350, 351]. Expression of heme oxygenase-1 in the glomerulus has not been reported, but *in vitro*, cultured mesangial cells express this enzyme abundantly in response to IL-1β [352]. Together with the fact that cytokines and reactive oxygen/nitrogen species are important pathogenic mediators in glomerulonephritis [147, 314, 353], heme oxygenase-1 may be induced in inflamed glomeruli and could participate in the modulation of inflammatory processes.

Antioxidant enzymes

Oxidant stress is involved in various diseases, including inflammation. It is supposed that ROI play important roles in the generation of a broad array of human and experimental glomerular diseases [147].

ROI released by local neutrophils and macrophages trigger glomerular cell death [337], degradation of ECM [47], formation of thrombi and, thereby, contribute to the generation of diseases [147]. ROI may induce massive proteinuria via causing glomerular sieving defect without apparent histologic abnormality [354]. ROI also participate in the activation of resident cells. This is due to the fact that a variety of signaling molecules are redox-sensitive [355, 356]. For example, in mesangial cells, ROI trigger calcium signaling and phosphorylation of tyrosine kinases and activate transcription factors including activator protein 1 (AP-1) and NF-κB [339, 357, 358]. ROI may therefore affect a diverse range of cell function, including proliferation, apoptosis, matrix metabolism, and production of inflammatory mediators [359, 360]. In particular, ROI stimulate the production of chemokines and the expression of adhesion receptors by resident cells [361, 362] and, thereby, attract circulating leukocytes at affected sites. Based on these data, endogenous antioxidant enzymes should be important as the self-defense machinery that prevents glomerular injury [363].

(1) Superoxide dismutases.

Mn-SOD and Cu/Zn-SOD are included in this category. These SODs promote dismutation of the superoxide anion, an immediate oxygen metabolite with a high biological activity, to yield hydrogen peroxide. Mn-SOD is inducible by a variety of stimuli, including ROI, endotoxin, and inflammatory cytokines. In contrast, the expression of Cu/Zn-SOD is constitutive [363, 364]. Studies using transgenic mice provided evidence that overexpression of either SOD confers resistance to oxidant-induced injury [365, 366].

Mn-SOD is expressed in isolated normal glomeruli and in cultured mesangial, endothelial, and epithelial cells [367–369]. The role of glomerular SOD has been extensively investigated in PAN nephrosis, an experimental model of minimal change disease. In this model, the administration of SOD effectively attenuated proteinuria and foot process fusion of podocytes [370, 371]. Experimental depletion of SOD by the administration of diethyldithiocarbamate aggravated the disease (abstract; Hara et al, *Proc XIth Int Congr Nephrol*, 1990, p 442),

indicating the protective role of endogenous SOD in PAN nephrosis.

The expression of Mn-SOD in cultured glomerular cells is up-regulated by hydrogen peroxide, heat-aggregated IgG, or IL-1 [369, 372, 373]. This implies that Mn-SOD is up-regulated during glomerulonephritis. Currently, however, information is very limited regarding the kinetics and roles of SOD in glomerular inflammation. One report showed that when SOD was administered intravenously in rats, nephrotoxic serum nephritis was attenuated [374].

(2) Catalase and glutathione peroxidase.

Catalase and glutathione peroxidase catalyze the decomposition of hydrogen peroxide to yield oxygen and water [147]. When overexpressed, either enzyme confers cellular resistance to oxidant-induced injury [375, 376].

Catalase and glutathione peroxidase are normally expressed in the glomerulus, and the expression is up-regulated by oxidant stress [367]. The administration of catalase is effective for the treatment of PAN nephrosis [371]. Experimental depletion of endogenous glutathione peroxidase by a selenium-deficient diet or administration of diethyl maleate aggravates PAN nephrosis (abstract; Miyai et al. *Proc XIth Int Congr Nephrol*, 1990, p 1442) [377]. These data suggest that endogenous catalase and glutathione peroxidase function as defending enzymes against oxidant-mediated glomerular injury. Of note, rats fed with a diet deficient in selenium and vitamin E develop glomerular disease characterized by proteinuria and reduced glomerular filtration rate [378]. This result implies a "housekeeping" role of glutathione peroxidase (and other antioxidant enzymes) in the maintenance of normal glomerular function.

(3) Heme oxygenases.

Heme oxygenase-1 and heme oxygenase-2 operate as antioxidants at two different stages. These enzymes decrease the levels of the potential pro-oxidants heme and heme proteins and increase tissue concentrations of the antioxidant, bile pigments [348]. The potential role of these enzymes in glomerular disease has been addressed in the section about heat shock proteins.

Protein phosphatases

Phosphorylation of signaling proteins plays a cardinal role in the regulation of a number of cellular processes. The reversible control over protein phosphorylation requires not only protein kinases but also protein phosphatases. In many cases, target proteins are phosphorylated at specific sites by one or more protein kinases, leading to their activation. Following the phosphorylation, the phosphates are subsequently removed by specific protein phosphatases, resulting in deactivation of the target proteins. This allows for reversible and tight control over the activity of signaling proteins and halts cellular activation [379]. In this regard, protein phosphatases may serve as a piece of intracellular defense mechanisms.

A number of protein phosphatases have been identified. These include protein-tyrosine phosphatases (PTPs), protein-serine/threonine phosphatases, and dual-specificity phosphatases. Currently, distribution, kinetics, and function of protein phosphatases in the glomerulus are largely unknown, but recent investigation has shed light on putative roles of some protein phosphatases. The family of mitogen-activated protein kinases (MAP kinases), including extracellular signal-regulated kinase (ERK), JNK, and p38 MAP kinase, plays a crucial part in cellular activation in response to a broad array of extracellular stimuli [380]. Dual-specificity protein phosphatases participate in dephosphorylation of MAP kinases and thereby attenuate cell proliferation and stress-induced gene expression [379]. In cultured mesangial cells, several dual-specificity phosphatases are constitutively expressed, that include MAP kinase phosphatase 1 (MKP-1), PAC1, and B23 [381]. Treatment of mesangial cells with vanadate, an inhibitor of dual-specificity phosphatases, triggers mitogenesis and growth factor production, suggesting a role of protein phosphatases in mesangial cell inactivation [382]. The expression of MKP-1 in cultured mesangial cells is up-regulated by ROI [383]. In the oxidant-mediated anti-GBM glomerulonephritis, expression of MKP-1 is rapidly induced in mesangial cells [383]. Under pathological situations, the up-regulated MKP-1 may function as a breaking mechanism that attenuates activation of glomerular cells.

Interestingly, several PTPs, including PTPλ, receptor-type PTP in the brain and kidney (RPTP-BK) and glomerular epithelial protein 1 (GLEPP1), are constitutively and predominantly expressed in podocytes [384–386]. These PTPs may function as "housekeeping brakes" for podocytes and might explain their resistance to mitogenic stimuli.

Cyclin kinase inhibitors

Proliferation of eukaryotic cells is positively controlled by a set of nuclear proteins, called cyclins and cyclin-dependent kinases (CDKs). Cyclins are periodically expressed during specific phases of the cell cycle, and bind to and activate specific CDKs. G1/S phase cyclins and CDKs are required for G1/S transition and DNA synthesis. G2/M phase cyclins are required for mitosis [387]. The activity of CDKs is negatively regulated by CKIs that bind to and inactivate specific CDK-cyclin complexes and thereby arrest the cell cycle [388]. There are two families of CKI: the Cip/Kip family, and the INK4 (inhibitor of cdk4) family. The former comprises $p21^{Cip1/Waf1/Sdi1/Cap20}$ (p21), $p27^{Kip1}$ (p27) and $p57^{Kip2}$, and the latter does p15, p16, p18, and p19 [387]. The Cip/Kip family members inhibit function of cyclin-CDK complexes in the G1 and S phases. The INK4 family members inhibit cyclin-CDK

complexes only in the G1 phase. In various cell types, the overexpression of P21, p27, or P57 is sufficient to induce cell cycle arrest in G1.

Quiescent mesangial cells constitutively express p27 *in vitro.* Shankland et al investigated the role of p27 in proliferation of cultured mesangial cells [389]. After stimulation with PDGF or basic fibroblast growth factor, mesangial cells exhibited a substantial decrease in the p27 levels. Consequently, there was a marked increase in expression of cyclin A and CDK2. The decline of p27 was prevented by TGF-β1 that suppressed mesangial cell proliferation. Furthermore, antisense oligonucleotides against p27 enhanced growth factor-induced cell proliferation, suggesting the importance of p27 as an endogenous inhibitor of mesangial cell growth *in vitro.*

Shankland et al further examined the expression of cell-cycle proteins *in vivo* in normal rat kidneys and kidneys subjected to anti-Thy 1 glomerulonephritis [390]. They found that, in normal glomeruli, p27 was highly expressed, whereas the expression of p21 was low. In the nephritic glomeruli, the induction of mesangial cell proliferation was associated with increased expression of cyclin A and CDK2 and transient reduction in p27 levels. During the recovery phase, the expression of p21 was up-regulated and maintained until at least day 10, whereas the depressed levels of p27 returned to the baseline levels within five days. This result suggests that p27 and p21 play distinctive roles; that is, the former maintains mesangial cells to be quiescent, and the latter inhibits ongoing proliferation.

The role of p27 in the glomerulus has been further investigated using p27-null mutant mice [391]. In the normal condition, the null mutant mice did not exhibit any abnormality in the glomerulus. However, when anti-GBM nephritis was induced, the mutant mice developed more severe disease—increased cell proliferation, apoptosis, and matrix accumulation—compared with wild-type mice [391]. This result suggested that p27 functions as a "safeguard molecule" of the glomerulus against inflammation.

In nephritic glomeruli, podocytes are relatively resistant to mitogenic stimuli compared with mesangial and endothelial cells. To determine the mechanisms responsible for the lack of podocyte proliferation *in vivo*, Shankland et al examined the expression of CKIs in passive Heymann nephritis, in which the podocyte is the target of complement-mediated injury [392]. Following antibody deposition and complement activation, marked up-regulation of p21 and p27 and subsequent inhibition of CDK2 activity were observed in podocytes.

Taken together, p21 and p27 function as endogenous brakes to inhibit or terminate proliferation of resident cells in response to pathogenic, growth stimuli.

STRATEGIES FOR REINFORCEMENT OF GLOMERULAR SELF-DEFENSE

Pharmacological agents that attenuate functions of platelets/coagulants, leukocytes, complement, and hemodynamic stress have been considered for therapeutic intervention in glomerular disease. As an alternative strategy, the reinforcement of "self-defense" may be useful for attenuation of glomerular injury. Several tactics are available for this purpose. These include preconditioning, pharmacological modulation, and genetic manipulations.

Preconditioning

A brief period of ischemia leads to resistance of various organs to subsequent, severe ischemia. Yoshioka et al reported that in the kidney ischemic preconditioning prevented a severe reduction in glomerular filtration rate caused by ischemia/reperfusion or intra-arterial infusion of hydrogen peroxide [367]. This protective effect was ascribed, at least in part, to the induction of glomerular antioxidant enzymes, including Mn-SOD, catalase, and glutathione peroxidase.

As shown in Table 2, a number of defending molecules are inducible in response to proinflammatory or anti-inflammatory cytokines. Strictly controlled administration of cytokines might also achieve substantial enhancement of the glomerular self-defense.

Pharmacological manipulation

Glucocorticoids are used for the first-line treatment of nephrotic syndrome, including minimal change disease in which oxidants are involved as pathogenic mediators. Ichikawa et al investigated the effect of glucocorticoid on the expression of antioxidant enzymes in the glomerulus. They found that, *in vitro*, glomerular endothelial and mesangial cells treated by methylpredonisolone exhibited increased activity and expression of Mn-SOD and catalase [368]. They also showed that glomeruli isolated from rats treated with methylpredonisolone had significantly higher activity of Mn-SOD, glutathione peroxidase, and catalase compared with control rats [393]. In PAN nephrosis, a model of minimal change disease, glucocorticoid-treated rats showed less impairment in renal function, lack of foot process fusion, and lower lipid peroxidation levels [393]. The therapeutic effect of glucocorticoids may be, in part, due to the enhancement of endogenous self-defense machinery that attenuates pathogenic action of ROI.

Genetic manipulation

The progress in gene transfer technology has established the scientific basis for genetic engineering of somatic tissues. During the past years, several strategies have been developed to introduce exogenous genes into

the adult kidney [394, 395]. Using gene transfer technology, it should be feasible to reinforce self-defense of the glomerulus. Importantly, the gene transfer strategy is widely applicable; that is, a majority of defending molecules listed in Tables 1 and 2 are the potential targets for genetic manipulation.

(1) Gene transfer approaches to the glomerulus.

The introduction of foreign genes into the glomerulus is feasible via the renal circulation. Tomita et al reported an *in vivo* gene transfer approach using the hemagglutinating virus of Japan (HVJ)-liposome method [396]. In this approach, DNA, nuclear protein, and lipid are conjugated to form liposomes. Purified HVJ is inactivated by ultraviolet light and fused with the liposomes. By injection of the HVJ-liposome–coupled plasmid into the renal artery, expression of transgene is observed in glomerular cells, especially in mesangial cells [397]. Using this method, Badr et al showed that overexpression of 15-LO significantly improved glomerular filtration rate and proteinuria in anti-GBM nephritis in rats [abstract; Yura et al, *J Am Soc Nephrol* 6:890, 1995]. Another gene transfer approach was reported by Heikkila et al using a replication-deficient adenoviral vector [398]. In this method, pig kidneys are continuously perfused *in vivo* or *ex vivo* with an adenovirus that introduces a marker gene. Intense expression of the transgene product was detectable in glomerular cells, mainly in podocytes [399].

As an alternative approach, *ex vivo* gene transfer system has been developed by our group using the genetically engineered mesangial cell as a vector for gene delivery [155]. In this method, mesangial cells are cultured from isolated glomeruli, introduced with a foreign gene *in vitro*, and transferred back into the kidney via the renal circulation. Using β-galactosidase as a marker, we demonstrated efficient, site-specific gene delivery to the glomerulus and sustained transgene expression [155]. Using this approach, it is feasible to enhance glomerular self-defense. For example, the introduction of cDNA encoding IL-1ra conferred glomerular insensitiveness to IL-1 [400]. The transfer of a cDNA coding for active TGF-β1 reduced mitogenesis and cytokine responses of the glomerulus [154]. Fouqueray et al reported that intraglomerular transfer of a mesangial cell vector expressing human IL-10 resulted in a modest decrease in proteinuria in anti-GBM glomerulonephritis (abstract; Fouqueray et al, *J Am Soc Nephrol* 7:1698, 1996).

The approach to reinforcement of self-defense mechanisms has obvious requirements, the most important being that the defense systems should be bolstered only at a time point that is beneficial and that will not interfere with a protective inflammatory response. Toward achieving this goal, tight control over the transgene expression will be essential.

(2) In vivo *control of transgene activity.*

Many defending molecules are inducible in response to a local inflammatory milieu. By combining gene transfer systems with inducible promoters, it is feasible to mimic the inducible property of endogenous defending molecules. Using the mesangial cell vector combined with the tetracycline-controlled transactivation system, we reported a reversible on–off system that achieves site-specific, *in vivo* control of transgene activity in the glomerulus [401]. By combining the mesangial cell vector with an "inflammation-responsive promoter," we further demonstrated automatic, *in vivo* induction of transgene expression in the glomerulus in response to local inflammatory stimuli [402]. These approaches would enable *in vivo* regulation of defender genes in which the transgene is required to be activated during inflammation and deactivated when the inflammation has subsided.

(3) "Adoptive defense": Delivery of transgene products via the circulation.

Increased defense potential of the glomerulus against complement-mediated injury may be achieved via adoptive transfer of GPI-anchored complement regulatory proteins. DAF and CD59 are GPI-anchored proteins that can be released from a cell membrane and reinserted into heterologous cell membranes without alteration of their biological function. Kooyman et al generated transgenic mice in which human DAF or CD59 was overexpressed on the surface of red blood cells. Using these mice, they demonstrated that functional DAF and CD59 are effectively transferred from red blood cells to the vascular endothelium [403]. Using this manipulation, McCurry et al showed that the intermembrane transfer of human DAF and CD59 effectively protected swine-to-primate cardiac xenografts from humoral injury [404]. This strategy may allow for the accumulation of complement regulatory proteins on the surface of glomerular endothelial cells without a direct gene transfer to the glomerulus.

Similar "adoptive defense" may be achieved in a different way. Recently, Zhu et al showed that a certain transgene product synthesized in the liver is effectively delivered to the glomerulus via the systemic circulation [405]. Isaka et al also reported that intramuscular transfer of a decorin cDNA led to accumulation of decorin protein in the glomerulus and attenuated matrix expansion in anti-Thy 1 glomerulonephritis.

ACQUISITION BY THE GLOMERULUS OF "ANTI-INFLAMMATORY STATUS": A LESSON FROM ACUTE ANTI-THY 1 GLOMERULONEPHRITIS

As mentioned earlier, the concept of "glomerular self-defense" occurred to us via the finding that glomeruli in the regeneration phase of acute anti-Thy 1 glomerulonephritis (day 7) showed blunted responses to proinflammatory stimuli. As shown in Figure 2A, when normal

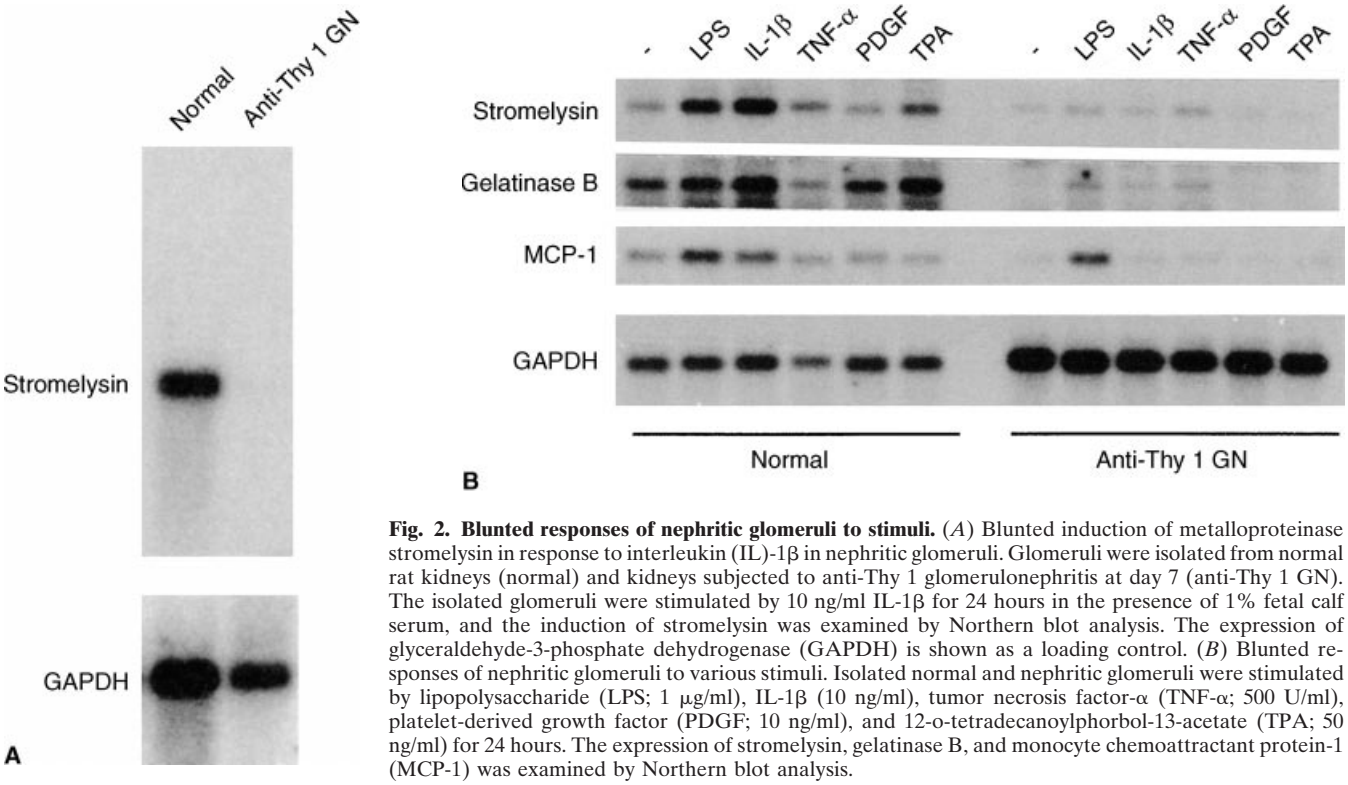

Fig. 2. Blunted responses of nephritic glomeruli to stimuli. (*A*) Blunted induction of metalloproteinase stromelysin in response to interleukin (IL)-1β in nephritic glomeruli. Glomeruli were isolated from normal rat kidneys (normal) and kidneys subjected to anti-Thy 1 glomerulonephritis at day 7 (anti-Thy 1 GN). The isolated glomeruli were stimulated by 10 ng/ml IL-1β for 24 hours in the presence of 1% fetal calf serum, and the induction of stromelysin was examined by Northern blot analysis. The expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is shown as a loading control. (*B*) Blunted responses of nephritic glomeruli to various stimuli. Isolated normal and nephritic glomeruli were stimulated by lipopolysaccharide (LPS; 1 μg/ml), IL-1β (10 ng/ml), tumor necrosis factor-α (TNF-α; 500 U/ml), platelet-derived growth factor (PDGF; 10 ng/ml), and 12-o-tetradecanoylphorbol-13-acetate (TPA; 50 ng/ml) for 24 hours. The expression of stromelysin, gelatinase B, and monocyte chemoattractant protein-1 (MCP-1) was examined by Northern blot analysis.

glomeruli were stimulated with the proinflammatory cytokine IL-1β, the expression of stromelysin was induced. Unexpectedly, however, the IL-1–triggered gene expression was blunted in the nephritic glomeruli. The blunted response was observed not only for stromelysin but also for other cytokine-inducible molecules, including gelatinase B and MCP-1. This insensitivity was consistently observed in the nephritic glomeruli triggered by other stimuli, including LPS and 12-o-tetradecanoylphorbol-13-acetate (TPA; Fig. 2B).

In the glomeruli seven days after the injection of the anti-Thy 1 antibody, mesangial cells are fully and preferentially activated, which suggests that defending molecules elaborated by mesangial cells may cause the blunted responses. At day 7 of anti-Thy 1 glomerulonephritis, macrophages are still present in the glomerulus [406]. Macrophage-derived factors might also participate in the reinforcement of glomerular self-defense. Based on these observations, several mechanisms can be postulated to explain the acquisition of the "anti-inflammatory status" by the nephritic glomeruli.

The first possibility is the elaboration of cytokine inhibitors such as IL-1ra. Although it has not been determined whether IL-1ra is expressed in anti-Thy 1 glomerulonephritis, monocytes and macrophages have the ability to produce IL-1ra abundantly. The insensitivity of the nephritic glomeruli to IL-1 may be ascribed to the elaborated IL-1ra.

The second possibility is that locally produced heparin-like molecules may inhibit activation of glomerular cells. Glomerular epithelial, endothelial, and mesangial cells have the ability to produce heparin-like substances [295, 296, 310, 311, 407]. In particular, mesangial cells produce HSPG in response to TGF-β [312], which is abundantly elaborated in anti-Thy 1 glomerulonephritis [38]. *In vivo*, increased production of HSPG is observed in anti-Thy 1 glomerulonephritis [408]. HSPG inhibits mesangial expression of immediate early genes, including c-*fos* and *egr-1* [299, 300], which are involved in the induction of cytokine-inducible genes. The insensitivity of the nephritic glomeruli to stimuli might be due to local accumulation of heparin-like molecules.

The third possibility is that certain signaling pathways may be selectively suppressed via protein phosphatases. For example, a recent report showed that MKP-1 is upregulated in the glomeruli of anti-Thy 1 glomerulonephritis [409]. MKP-1 suppresses activity of the MAP kinase family of molecules including ERK, JNK, and p38 MAP kinase [410, 411]. MAP kinases are generally involved in signaling pathways to transcriptional induction of various genes in response to inflammatory cytokines, growth factors, LPS, and pharmacological stimuli such as TPA [380]. The induction of protein phosphatases, including MKP-1, might be responsible for the attenuated responses of the nephritic glomeruli to external stimuli.

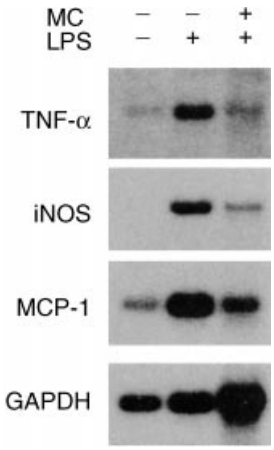

Fig. 3. Inactivation of macrophages by bystander mesangial cells. Normal rat macrophages (NR8383) were seeded on plastic or on confluent culture of rat mesangial cells (MC). After six hours, cells were stimulated by LPS (1 μg/ml) for 14 hours. Then the macrophages were isolated from the cultures and subjected to Northern blot analyses of activation markers: TNF-α, inducible nitric oxide synthase (iNOS) and MCP-1.

An interesting finding is that the nephritic glomeruli containing many macrophages exhibit blunted responses to LPS and TPA, the strong activators for monocytes/macrophages. It indicates that, although macrophages are present, they become deactivated and thus insensitive to stimuli within the inflammatory milieu in which mesangial cells are activated. Cultured mesangial cells have the ability to deactivate macrophages [134, 135]. Figure 3 shows the LPS responses of rat macrophages cocultured with rat mesangial cells. The cocultured macrophages exhibit blunted expression of TNF-α, iNOS, and MCP-1 in response to LPS. Together with the fact that a number of defending molecules are produced by mesangial cells (Tables 1 and 2), this result raises the possibility that the mesangial cell is the pivotal defender of the glomerulus that functions against macrophage-mediated injury.

What kind of molecules do the nephritic glomeruli mobilize to inactivate macrophages? In this regard, macrophage-deactivating cytokines, including IL-4, IL-6, IL-10, IL-13 and TGF-β, may play a role. Currently, information is limited regarding the expression of IL-4, IL-6, IL-10, and IL-13 in anti-Thy 1 nephritis, but it is well known that active TGF-β1 is abundantly produced in this nephritis model [38]. Several reports provided evidence that TGF-β1 inhibits production of IL-1β, TNF-α, and MCP-1 by activated macrophages [134, 144, 146]. TGF-β1 is a possible candidate that inactivates local macrophages.

Using a technique for *in vivo* macrophage transfer, it has been shown that glomerular cells in anti-Thy 1 glomerulonephritis are insensitive to the triggering action of activated macrophages (abstracts; Ogura et al, ibid; Sütö and Kitamura, ibid) [1, 2]. Cultured mesangial cells, glomerular endothelial cells, and isolated glomeruli treated with TGF-β1 exhibit depressed responses to IL-1β and TNF-α (abstract; Danoff and Jiang, ibid) [154, 159, 160]. TGF-β1-transfected mesangial cells show a blunted response to IL-1β, whereas mesangial cells expressing the mutant TGF-β receptor show an enhanced response to IL-1, indicating that TGF-β1 functions as an autocrine inhibitor of the certain cytokine response in glomerular cells [1]. These data point to the potential of local TGF-β1 for suppression of acute inflammatory responses in the glomerulus via diminishing activity of both inflammatory macrophages and resident glomerular cells.

The anti-Thy 1 model (one shot model) mimics mesangial proliferative glomerulonephritis in humans, but the pathological changes are transient and reversible. After several weeks, the lesions spontaneously disappear without irreversible sclerosis or scarring. The anti-Thy 1 glomerulonephritis should therefore be considered as a model of glomerular repair, but not progressive glomerulosclerosis or scarring. During repair of the mesangiolytic glomerulonephritis, transient accumulation of ECM seems to be essential. This is because *in vivo* inactivation of TGF-β1 attenuates the accumulation of glomerular ECM, whereas it disturbs repair of mesangiolysis caused by anti-Thy 1 antibodies (Dr. Seiya Okuda, Kurume University School of Medicine, personal communication). It raises the possibility that TGF-β1 functions as an endogenous repair factor, rather than as a prosclerotic mediator, in this nephritis model. The "blackguard molecule" TGF-β1 should be rehabilitated under certain pathologic situations.

Currently, the contribution of NO and anti-inflammatory eicosanoids, the potential inactivators of leukocytes and glomerular cells, is undetermined. It is also unknown whether the insensitivity of nephritic glomeruli to stimuli is observed in other experimental glomerulonephritis. Extensive investigation is required to work out the molecular jigsaw of the glomerular self-defense system in individual human and experimental glomerular disease.

PERSPECTIVE

The balance between external offending factors and internal defense machinery determines the fate of glomerular disease. A wide array of investigations have been pursued during the past years especially focusing on the offensive side of diseases. In contrast, only little attention has been paid to its defensive side. Information is still limited regarding what kind of endogenous molecules counteract complement, platelets, leukocytes and inflammatory mediators, and how their expression is controlled under pathophysiologic circumstances. Interestingly, some defending molecules are generated in response to their specific targets. For example, oxidants, IL-1, PDGF, TGF-β, and complement trigger expression of their spe-

cific inhibitors—antioxidants, IL-1ra, SPARC, decorin, and complement regulatory proteins—in resident cells, respectively. Elucidation of this sophisticated, autoregulatory mechanism may be important for a better understanding of the glomerular defense system. One defending molecule often triggers the expression of other defenders. For example, TGF-β induces IL-10, IL-1ra, SPARC, TIMP, HSPG, decorin, CD59, and CKIs (p15, p21, p27). IL-6 triggers the production of IL-1ra, sTNFR, PGE_2 and PGI_2. IL-10 up-regulates IL-1ra and sTNFR. IL-4 and IL-13 stimulate IL-1ra, IL-1 decoy receptor and 15-LO expression. Heparin and HSPG induce endothelial NO, TFPI, HSPG, and potentiate function of C1 inhibitor, TGF-β, and Mn-SOD. Intermolecular defense cascades and networks in the glomerulus are other important issues to be addressed in the future.

In addition to complement, platelets, leukocytes, and inflammatory mediators, vasoconstrictive substances are also considered pathogenic factors involved in glomerular injury [412]. Mechanical stress associated with local hypertension induces glomerular injury directly or indirectly [413]. In this regard, endogenous vasodepressors including NO and certain eicosanoids may form a line of self-defense [202, 314]. In this article, we have not dealt with hemodynamic-associated glomerular injury, but it should be another field in which self-defense is proposed to maintain glomerular integrity.

The establishment of effective therapeutic approaches to glomerular disease is still under way. Pharmacological inhibition of local pathogenic components is a possible approach. Alternatively, the reinforcement of endogenous defense mechanisms by pharmacologic and/or genetic manipulations may provide novel avenues to the treatment of glomerular disease. Efficient and realistic therapy would be feasible only if molecular events underlying glomerular diseases are fully elucidated from both the offensive and defensive sides.

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APPENDIX

Abbreviations used in this article are: AP-1, activator protein 1; α-SMA, α-smooth muscle actin; ATP/ADPase, adenosine triphosphatase and adenosine diphosphatase; AVP, arginine vasopressin; CDK, cyclin-dependent kinase; CKI, cyclin kinase inhibitor; CR1, complement receptor 1; Crry, complement receptor related gene y; DAF, decay accelerating factor; ECM, extracellular matrix; EC-SOD, extracellular superoxide dismutase; EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; GBM, glomerular basement membrane; G-CSF, granulocyte colony stimulating factor; GLEPP1, glomerular epithelial protein 1; GM-CSF, granulocyte macrophage colony-stimulating factor; GPI, glycosyl-phosphatidylinositol; HSP, heat shock protein; HSPG, heparan sulfate proteoglycan; HVJ, hemagglutinating virus of Japan; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; IL-1ra, IL-1 receptor antagonist; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; LIF, leukemia inhibitory factor; LO, lipoxygenase; LPS, lipopolysaccharide; LX, lipoxin; MAC, membrane attack complex; MAP kinase, mitogen-activated protein kinase; MCP, membrane cofactor protein; MCP-1, monocyte chemoattractant protein-1; MIF, macrophage migration inhibitory factor; MIP-1α, macrophage inflammatory protein 1α; MIP-2, macrophage inflammatory protein-2; MKP-1, MAP kinase phosphatase 1; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; NO, nitric oxide; p21, $p21^{Cip1/Waf1/Sdi1/Cap20}$; p27, $p27^{Kip1}$; PAN, puromycin aminonucleoside; PDGF, platelet-derived growth factor; PG, prostaglandin; PTP, protein-tyrosine phosphatase; PUMP, punctuated metalloproteinase; ROI, reactive oxygen intermediates; RPTP-BK, receptor-type PTP in the brain and kidney; SPARC, secreted protein acidic and rich in cysteine; sTNFR, soluble tumor necrosis factor receptor; TFPI, tissue factor pathway inhibitor; TGF-β, transforming growth factor-β; Th2, T cell helper type 2; TIMP, tissue inhibitor of metalloproteinases; TNF-α, tumor necrosis factor-α; t-PA, tissue-type plasminogen activator; TPA, 12-o-tetradecanoylphorbol-13-acetate; u-PA, urokinase-type plasminogen activator.

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